Book of abstracts

and Program Agenda

XVIII

National Plant Biochemistry and Molecular Biology Congress

XI Symposium México-USA

1st ASPB México Section Meeting

28 - 31 October, 2019
Mérida, Yucatán, México

"XVIII National Plant Biochemistry and Molecular Biology Congress - XI Symposium México-USA & 1st. ASPB Mexico Section Meeting"

XVIII National Plant Biochemistry and Molecular Biology Congress

XI Symposium México-USA

1st ASPB México Section Meeting

Monday, Oct 28

Maya Hall

15:00 18:00 — *Registration*

Tulum & Kabah Hall

18:15 18:30 — *Welcome & Opening*

18:30 19:30 — *Keynote Lecture*
Virginia Walbot
"Specification and differentiation of meiotic and somatic cells in maize anthers"

Patio Central

19:30 21:00 — *Welcome Cocktail*

Tuesday, Oct 29

Genetic improvement, from natural variation to new technologies

Tulum & Kabah Hall

08:30 09:00 — Fernando Rivadavia - Coffee Talk Illumina-Analitek
"Genomic selection & Genotyping technologies"

09:00 09:30 — Donald Richard Ort
"Improving photosynthetic efficiency for improved crop yield"

09:30 10:00 — June K. Simpson Williamson
"Unlocking the potential of Agave species as sustainable crops for marginal lands under a changing climate"

10:00 10:30 — Blake Meyers
"PhasiRNAs as modulators of traits in plants"

10:30 11:00 — *Coffee Break*

11:00 11:30 — Daniel Harrison Chitwood
"Topological Data Analysis (TDA) as a method to comprehensively measure the plant form"

11:30 12:00 — César Petroli
"Genetic diversity in world's largest wheat collection may hold the key to food security"

12:00 12:30 — Carlos Humberto Ortíz Ramírez
"A new model of root development revealed by CRISPR and Single-Cell RNA Seq"

12:30 13:00 — *Flash Talks*

13:00 14:30 — *Lunch*

	1 Development and organ specification Moderator: Nayelli Marsch Tulum Hall	2 Signal Transduction Moderator: Marina Gavilanes Kabah Hall
14:30 14:50	Escobar-Tovar, L. *"Signals back and forth: the epistasis of plastid translation and the ApoCarotenoid Signal 1 (ACS1) in leaf development"*	Ek-Ramos, M.J. *"Microbial endophytes change phytohormone levels for drought and plant pathogens tolerance"*
14:50 15:10	Durán, Y. *"BOL modulates gynoecium development and its cytokinin response"*	Ponce-Pineda, I.G. *"Winter winner, energy dinner. H + -ATPase activity regulation through MAP kinases during cold conditions"*
15:10 15:30	Shishkova, S. *"Uncovering the genetic regulation of determinate root growth in Cactaceae"*	Saucedo-García, M. *"Trichoderma asperellum protects tomato plants against fungal infections diseases through inhibition of reactive oxygen species production"*
15:30 15:50	Alatorre-Cobos, F. *"Genomics of cellulose and lignin biosynthesis in Agave tequilana Weber"*	Rosas-Santiago P. *"Cornichon sorting and regulation of GLR channels underlie pollen tube Ca 2+ homeostasis"*
15:50 16:10	Abraham-Juárez M.J. *"Differential expression and interaction profiles of maize MADS-box dimer evolutionary variants"*	Zoghbi-Rodríguez, N. *"Analysis of cell signaling in Capsicum chinense response to pathogens: a vision of molecular dynamics in plant defense"*
16:10 16:30	Torres-Martínez H.H. *"Lateral root formation in Arabidopsis thaliana starts from a single founder cell: new insights into initiation process"*	Cruz García, F. *"Self-incompatibility: a way for mothers to prevent a progeny with low fitness"*

16:30 17:00 — *Coffee Break* (Maya Hall: *Having Coffee with...*)

Uxmal Hall

17:00 19:00 — *Posters - Odd numbers*

Tulum & Kabah Hall

19:00 20:00 — *Keynote Lecture*
Stefan De Folter
"The integration of transcription factor and hormone signalling functions during gynoecium development"

Wednesday, Oct 30

Plant Development & Phenomics

Tulum & Kabah Hall

08:30 09:00 — Mario Muñoz - Olympus America
"Innovations in Olympus Confocal Microscopy"

09:00 09:30 — Michael J. Scanlon
"How leaves grow wide: NARROWSHEATH1 controls mediolateral outgrowth of lateral organs"

09:30 10:00 — Jaimie M. Van Norman
"IRK, a transmembrane receptor kinase, is polarly localized and represses root ground tissue cell divisions"

10:00 10:30 — Argelia Lorence
"Leveraging Phenomic Approaches to Accelerate the Development of More Resilient Crops"

10:30 11:00 — *Coffee Break*

11:00 11:30 — Mario Arteaga
"DICER-mediated Reprogramming of Cell Fate Specification in Marchantia polymorpha"

11:30 12:00 — David Jackson
"An unexpected role of trehalose phosphate phosphatases in meristem determinacy"

12:00 12:30 — Ulises Rosas
"Genetic basis of domesticated traits in date palm (Phoenix dactylifera)"

12:30 13:00 — *Flash Talks*

13:00 14:30 — *Lunch*

	3 Regulation of Gene expression Moderator: Enrique Castaño Tulum Hall	4 Plant-Microbe Interactions Moderator: Georgina Hernández Kabah Hall
14:30 14:50	Castaño E. *"Evolution and function of fibrillarin in plants"*	Hernández G. *"The common bean –Rhizobium etli Nitrogen Fixing Symbiosis: deciphering novel regulatory pathways"*
14:50 15:10	Jiménez-Morales, E. *"A singular genetic trick in the course of evolution protects Arabidopsis thaliana from drought stress"*	Nova-Franco, B. *"A WRKY transcription factor required for nodule development and symbiotic nitrogen fixation in Medicago truncatula"*
15:10 15:30	Juárez-González, VT. *"Little-Big Guardians of Dedifferentiation: sRNA impact on Maize Somatic Embryogenesis"*	Oswaldo Valdés-López *"Early Phosphorylated Protein1: A Novel Positive Regulator of the legume-rhizobia symbiosis"*
15:30 15:50	Romo-Avalos A. *"Functional analysis of geminivirus promoters for overexpression of recombinant proteins of pharmaceutical interest in Chlamydomonas reinhardtii"*	Solís-Miranda, J. *"Exploring the roles of the RALF-FER-RIPK signaling during the symbiosis of common bean with rhizobia"*
15:50 16:10	Brieba L.G. *"Plant mitochondrial DNA replication: between recombination and origin dependent replication mechanisms mediated by unique enzymes"*	Camarena-Pozos *"Microbial volatile organic compounds in desert plants: identification, function and biotechnological application"*
16:10 16:30	Garza-Aguilar SM *"The complexity of folate polyglutamylation in plants: ripening and ethylene modulate polyglutamylated profiles in climacteric fruits plus systematic analysis of the glutamyl tail-editing enzymes"*	Morales-Ruiz, E. *"Study of a plant – pathogen – endophyte interaction: the case of maize – Fusarium verticillioides – Bacillus cereus B25"*
16:15 16:30		Puch-Hau, C. *"Unveiling the microbiome of Haplaxius crudus: the coconut lethal yellowing phytoplasma vector"*

16:30 17:00 — *Coffee Break*

Uxmal Hall

17:00 19:00 — *Posters - Even numbers*

Tulum & Kabah Hall

19:00 20:00 — *Keynote Lecture*
Jen Sheen
"Probing the universal energy-stress signaling network"

Thursday, Oct 31

Plant stress responses: common challenges for the US and Mexico

Tulum & Kabah Hall

09:00 09:30 — Ramiro Lascano
"Plant environment interactions: the redox connection and autophagy involvement"

09:30 10:00 — Patricia Coello
"Participation of SnRK1 kinases in plant energy signaling"

10:00 10:30 — Rafael Rivera Bustamante
"Capsicum-geminivirus interaction: Comparison of two resistance mechanisms"

10:30 11:00 — *Coffee Break*

11:00 11:30 — Katayoon Dehesh
"How Interorganellar communication regulates plant stress responses"

11:30 12:00 — Mario Serrano
"Rare-earth elements and their role in the battle between plants and Botrytis cinerea"

12:00 12:30 — César Luis Cuevas Velázquez
"Development of plant intrinsically disordered proteins-based fluorescence biosensors to track the osmotic environment"

12:30 14:30 — *Lunch*

	5 Stress responses and climate change Moderator: Gladys Cassab Tulum Hall	6 Epigenetics Moderator: Jose Luis Reyes Kabah Hall
14:30 14:50	Bernal, L. *"Carbon metabolism in bean pods during seed development"*	Trejo-Arellano, M.S. *"The Arabidopsis methylome is robust to dark-induced senescence"*
14:50 15:10	Mery Nair Saenz *"Analysis of the hydrotropic root response and elongation of mesocotyl in DTMA (Drought Tolerant Maize for Africa) hybrids under low water potential gradient conditions"*	Clelia De la Peña *"The epigenetic behind the albinism in Agave"*
15:10 15:30	Dorantes-Acosta, A. *"Molecular and ecophysiological characterization of abiotic stress responses in Marchantia polymorpha"*	Reyes JL *"RNA methylation or the epi-transcriptomic control of gene expression in the moss Physcomitrella patens"*
15:30 15:50	Cassab, G.I. *"Analysis of genetic variation associated to hydrotropism and deep planting resistance in maize (Zea mays L.) revealed genes controlling response to drought, growth, development and adaptation to global heating"*	Noé Durán Figueroa *"Female gametogenesis, a pathway mediated by MIR822 regulates germline linage in Arabidopsis thaliana"*
15:50 16:10	José López Bucio *"Chromate treatment of MEDIATOR mutants triggers the splitting of the root meristem in Arabidopsis"*	Arnaud Ronceret *"Biotechnological implications of the analysis of plant meiotic mutants"*
16:10 16:30	Trujillo-Pahua V *"Metabolic response to larval herbivory in Physalis sp."*	González-Morales SI *"ptxD/Phi as a dominant and stable selectable marker system for cyanobacteria and microalgae"*

16:30 17:00 — *Coffee Break*

Tulum & Kabah Hall

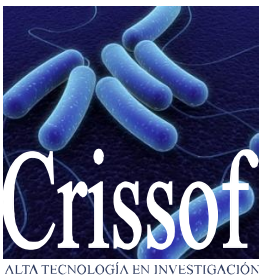
17:00 17:45 — *Business Meeting*

17:45 18:45 — *Keynote Lecture*
Alejandra Covarrubias
"The relevance of carbon distribution towards reproductive organs in the resistance to terminal drought of common bean cultivars"

18:45 19:00 — *Closure & Awards*

21:00 23:59 — *Gala Dinner*

XVIII National Plant Biochemistry and Molecular Biology Congress
XI Symposium México-USA & 1st ASPB Mexico Section Meeting



XVlll National Plant Biochemistry and Molecular Biology Congress

XI Symposium México-USA & 1st ASPB Mexico Section Meeting

Welcome!

The organizing committee is pleased to welcome all the participants to Mérida for the XVlll National Plant Biochemistry and Molecular Biology Congress, XI Symposium México-USA & 1st ASPB Mexico Section Meeting.

This meeting takes place biannually in order to present and discuss new scientific advances in Plant Molecular Biology and to promote collaborations on today's major questions in the field. This meeting is already an important tradition for the Plant Biochemistry and Molecular Biology branch of the Mexican Biochemistry Society (Sociedad Mexicana de Bioquímica, SMB) and the USA plant community. In addition, we are particularly pleased that this year the 1st Mexico Section of the American Society of Plant Biologists (ASPB) also takes place. We are convinced that both the growth and strength of Plant Sciences communities and the interactions between Mexican and US scientists is of paramount importance in addressing important challenges common to both countries. Therefore, the 1st ASPB Mexico Section Meeting will deepen these relations and will open new avenues of collaboration and exchange between countries and scientific societies.

We are also pleased that this meeting has an international flavor because, in addition to our USA colleagues, we have participants from Latin America (Chile, Brazil, Uruguay, Argentina, Ecuador, and Puerto Rico), Europe (United Kingdom, Germany, France, The Netherlands, Sweden, Belgium, Czech Republic, Spain, and Portugal), Australia, and Japan.

The meeting takes place in the beautiful city of Mérida which has a colorful history, and is the home of impressive archeological sites that are landmarks of Mexican culture.

XVlll National Plant Biochemistry and Molecular Biology Congress
XI Symposium México-USA & 1st ASPB Mexico Section Meeting

The organizing committee of this event is deeply grateful to all who helped in the preparation of the scientific program and to all the speakers for accepting our invitation unconditionally.

Welcome all the participants!!! We think we have a fabulous program and we hope that you enjoy the meeting and your stay in Mérida. Let us together celebrate Plant Science!!!

Organizing committee 2019:

Patricia León	Instituto de Biotecnología, UNAM, Mexico
Tzvetanka Dinkova	Facultad de Química, UNAM, Mexico
Alfredo Herrera Estrella	LANGEBIO, CINVESTAV, Mexico
Ruben Rellan	North Carolina State University, USA
Luis Cárdenas	Instituto de Biotecnología, UNAM, Mexico
Teresa Hernández Sotomayor	Centro de Investigación Científica de Yucatán (CICY), Mexico
Sally Mackenzie	Pennsylvania State University, USA
Joseph G. Dubrovsky	Instituto de Biotecnología, UNAM, Mexico

Program Agenda

MONDAY OCTOBER 28

15:00 – 18:00 **Registration**

18:15 – 18:30 **Welcome and Opening**

18:30 – 19:30 **Keynote lecture:**
Specification and differentiation of meiotic and somatic cells in maize anthers.
Walbot, V. *(page 2)*
Moderator: Patricia Leon.

19:30 – 21:00 **Welcome Cocktail**

TUESDAY OCTOBER 29

GENETIC IMPROVEMENT, FROM NATURAL VARIATION TO NEW TECHNOLOGIES
Moderator: June Simpson

08:30-09:00 **Illumina – Analitek Coffee Talk: Genomic selection & Genotyping technologies**
Rivadavia, F.

09:00 – 09:30 **Improving Photosynthetic Efficiency for Improved Crop Yield.**
South, P., Cavanagh, A., Liu, H., Ort, D. *(page 7)*

09:30 – 10:00 **Unlocking the potential of *Agave* species as sustainable crops for marginal lands under a changing climate.**
Simpson, J. *(page 8)*

10:00 – 10:30 **PhasiRNAs as modulators of traits in plants.**
Meyers, B. *(page 9)*

10:30 – 11:00 **Coffee Break**

11:00 – 11:30 **Topological Data Analysis (TDA) as a method to comprehensively measure the plant form.**
Chitwood, D. *(page 10)*

11:30 – 12:00 **Genetic diversity in world's largest wheat collection may hold the key to food security.**
Sansaloni, C., Franco, J., Santos, B., Singh, S., Petroli, C., Campos, J., Dreher, K., Payne, T., Marshall, D., Kilian, B., Milne, I., Raubach, S., Shaw, P., Stephen, G., Carling, J., Saint Pierre, C., Burgueño, J., Crosa, J., Kehel, Z., Amri, A., Kilian, A. , Wenzl, P., Caccamo, M., Pixley, K. *(page 11)*

12:00 – 12:30 **A new model of root development revealed by CRISPR and Single-Cell RNA Seq.**
Ortiz-Ramírez, C., Demesa-Arevalo, E., Xu, X., Gingeras, T., Jackson, D., Gallagher K., Birnbaum, K. *(page 12)*

12:30 – 13:00 **Flash talks**

13:00 – 14:30 **Lunch**

CONCURRENT SESSIONS

MINISIMPOSIUM 1: DEVELOPMENT AND ORGAN SPECIFICATION
Moderator: Nayelli Marsch

14:30 – 14:50 **Signals back and forth: the epistasis of plastid translation and the ApoCarotenoid Signal 1 (ACS1) in leaf development**
Escobar-Tovar, L., Sierra, J., Hernández-Muñoz, A., Mathioni, S., Cordoba, E., McQuinn, R.P., Meyers, B., Pogson, B., León, P. *(page 26)*

14:50 – 15:10 **BOL modulates gynoecium development and its cytokinin response**
Durán, Y., Serwatowska, J, Reyes, I., de Folter, S., Marsch, N. *(page 27)*

15:10 – 15:30 **Uncovering the genetic regulation of determinate root growth in Cactaceae**
López-Valle, M., Rodriguez-Alonso, G., Formey, D., Matvienko, M., Napsucialy-Mendivil, S., Dubrovsky, J., Shishkova, S. *(page 28)*

15:30 – 15:50 **Genomics of cellulose and lignin biosynthesis in Agave tequilana Weber**
Alatorre-Cobos, F., Maceda-López, L. F., Ibarra-Laclette, E. Ávila de Dios, E., Moran-Velázquez, D. C., Villalpando-Aguilar, J. L., López-Pérez, M., Góngora-Castillo, E. B., Simpson, J. *(page 29)*

15:50 – 16:10 **Differential expression and interaction profiles of maize MADS-box dimer evolutionary variants**
Abraham-Juárez M.J., Schrager-Lavelle A., Man J. and Bartlett M. *(page 30)*

16:10 – 16:30 **Lateral root formation in Arabidopsis thaliana starts from a single founder cell: new insights into initiation process**
Torres-Martínez H.H., Hernández-Herrera P., Corkidi-Blanco G., Dubrovsky J. G. *(page 31)*

MINISIMPOSIUM 2: SIGNAL TRANSDUCTION
Moderator: Marina Gavilanes Ruiz

14:30 – 14:50 **Microbial endophytes change phytohormone levels for drought and plant pathogens tolerance.**
Ek-Ramos, M.J., Castillo-López, D., Sword, G.A. *(page 32)*

14:50 – 15:10 **Winter winner, energy dinner. H^+-ATPase activity regulation through MAP kinases during cold conditions.**
Ponce-Pineda, I.G., González-Córdova, C.D., Carmona-Salazar, L., Saucedo-García, M., Guevara-García, A. A., Cahoon, E., Cahoon, E.B., Gavilanes-Ruiz, M. *(page 33)*

15:10 – 15:30 ***Trichoderma asperellum* protects tomato plants against fungal infections diseases through inhibition of reactive oxygen species production.**
Herrera-Téllez, VI., Cruz-Olmedo, A.K., Plasencia, J., Gavilanes-Ruíz, M., Arce-Cervantes, O., Hernández-León, S., Saucedo-García, M. *(page 34)*

15:30 – 15:50 **Cornichon sorting and regulation of GLR channels underlie pollen tube Ca^{2+} homeostasis.**
Rosas-Santiago, P., Wudick M.M., Portes M., T., Michard E., Lizzio M. A., Oliveira-Nunes C., Campos C., Santa-Cruz D.D., Carvalho J. C., Lima P. T., Pantoja O., Feijó J. A. *(page 35)*

15:50 – 16:10 **Analysis of cell signaling in *Capsicum chinense* response to pathogens: a vision of molecular dynamics in plant defense.**
Zoghbi-Rodríguez, N., Sánchez-Sandoval, E., González-Mendoza, V., Cab-Guillén, Y., Muñoz-Sánchez, J., González Estrada, T., Hernández-Sotomayor, S.M.T. *(page 36)*

16:10 – 16:30 **Self-incompatibility: a way for mothers to prevent a progeny with low fitness.**
Cruz-García, F., Cruz-González, Y., Torres-Rodríguez, D., Nájera-Torres, E. *(page 37)*

16:30 – 17:00 **Coffee Break**

17:00 – 19:00 **Posters: Odd numbers** **(see Index of posters on pages 282-284)**

19:00 – 20:00 **Keynote lecture:**
The integration of transcription factor and hormone signalling functions during gynoecium development.
De Folter, S. *(page 3)*
Moderator: Luis Cardenas.

WEDNESDAY OCTOBER 30

PHENOMICS AND DEVELOPMENT
Moderator: Joseph Dubrovsky

08:30 09:00 **Olympus America: Innovations in Olympus Confocal Microscopy**
Muñoz, M.

09:00 – 09:30 **How leaves grow wide: *NARROWSHEATH1* controls mediolateral outgrowth of lateral organs.**
Conklin, P. A., Conlon, B., Shimizu, R., Johnston, R.M, Scanlon, M.J. *(page 13)*

09:30 – 10:00 **IRK, a transmembrane receptor kinase, is polarly localized and represses root ground tissue cell divisions.**
Van Norman, J.M., Campos, R., Goff, J. *(page 14)*

10:00 – 10:30 **Leveraging phenomic approaches to accelerate the development of more resilient crops.**
Lorence, A. *(page 15)*

10:30 – 11:00 **Coffee Break**

11:00 – 11:30 ***DICER*-mediated Reprogramming of Cell Fate Specification in *Marchantia polymorpha*** Aguilar-Cruz, A., Dorantes-Acosta, A., Oltehua-López, O., Kohchi, T., Ishizaki, K., Bowman, J., Grimanelli, D., Haseloff, J., Arteaga-Vázquez, M. *(page 16)*

11:30 – 12:00 **An unexpected role of trehalose phosphate phosphatases in meristem determinacy.**
Claeys, H., Vi, S.L., Xu, X., Satoh-Nagasawa, N., Eveland, A.L., Goldshmidt, A., Feil, R., Beggs, G.A., Sakai, H., Brennan, R.G., Lunn, J.E., Jackson, D. *(page 17)*

12:00–12:30 **Genetic basis of domesticated traits in date palm (Phoenix dactylifera).**
Hazzouri, K.M., Gros-Balthazard, M., Flowers, J.M., Ferrand, S., Fresquez, Z., Rosas U., Purugganan, M.D. *(page 18)*

12:30 – 13:00 **Flash talks**

13:00 – 14:30 **Lunch**

CONCURRENT SESSIONS

MINISIMPOSIUM 3: REGULATION OF GENE EXPRESSION
Moderator: Enrique Castaño

14:30 – 14:50 **Evolution and function of fibrillarin in plants**
Castaño, E., Pereira, A., Gonzalez, W. *(page 38)*

14:50 – 15:10 **A singular genetic trick in the course of evolution protects *Arabidopsis thaliana* from drought stress.**
Jiménez-Morales, E., Aguilar-Hernández, V., Aguilar-Henonin, L., Guzmán, P. *(page 39)*

15:10 – 15:30 **Little-Big Guardians of Dedifferentiation: sRNA impact on Maize Somatic Embryogenesis**
Juárez-González, V.T., Dinkova, T.D. *(page 40)*

15:30 – 15:50 **Functional analysis of geminivirus promoters for overexpression of recombinant proteins of pharmaceutical interest in *Chlamydomonas reinhardtii***

Romo-Avalos, A., Reyes-Barrera, K., Avalos-Calleros, J., Alpuche-Solis, A., Argüello-Astorga, G. *(page 41)*

15:50 – 16:10 **Plant mitochondrial DNA replication: between recombination and origin dependent replication mechanisms mediated by unique enzymes**
Brieba, L.G. *(page 42)*

16:10 – 16:30 **The complexity of folate polyglutamylation in plants: ripening and ethylene modulate polyglutamylated profiles in climacteric fruits plus systematic analysis of the glutamyl tail-editing enzymes**
Garza-Aguilar, S.M., García-Salinas, C., Mejía-Ponce, P.M., Licona-Cassani, C., Ramos-Parra, P., Díaz de la Garza, R.I. *(page 43)*

MINISIMPOSIUM 4: PLANT - MICROBE INTERACTIONS
Moderator: Georgina Hernández

14:30 – 14:50 **The common bean –*Rhizobium etli* Nitrogen Fixing Symbiosis: deciphering novel regulatory pathways**
Hernández, G., Formey, D., Ramírez, M., Leija, A., Fuentes, S., Ayra, L., Martín-Rodríguez, J., Íñiguez, L. *(page 44)*

14:50 – 15:10 **A *WRKY* transcription factor required for nodule development and symbiotic nitrogen fixation in *Medicago truncatula***
Nova-Franco, B., Liu, W., Sparks, M., Kolape, J., Sinharoy, S., Udvardi, M. *(page 45)*

15:10 – 15:30 **Early Phosphorylated Protein1: A Novel Positive Regulator of the legume-rhizobia symbiosis**
Valdés-López, O., Delaux, P.M., Ané, J.M., Isidra-Arellano, M.C., Ferrer-Orgaz, S., Rodríguez-Pozas, E., Casarrubias-Sandoval, A., Sánchez-Correa, M.S., Reyereo-Saavedra, M.R. *(page 46)*

15:30 – 15:45 **Exploring the roles of the *RALF-FER-RIPK* signaling during the symbiosis of common bean with rhizobia**
Solís-Miranda, J., Juárez-Verdayes, M., Nava N., Leija, A., Quinto, C. *(page 47)*

15:45 – 16:00 **Microbial volatile organic compounds in desert plants: identification, function and biotechnological application**
Camarena-Pozos, D.A., Flores-Núñez, V.M., López, M.G., López-Bucio, J., Partida-Martínez, L.P. *(page 48)*

16:00 – 16:15 **Study of a plant – pathogen – endophyte interaction: the case of maize – *Fusarium verticillioides* – *Bacillus cereus B*25**
Morales-Ruiz, E., Sánchez-Valle, V., Báez-Astorga P. A., Maldonado-Mendoza, I. E. *(page 49)*

16:15 – 16:30 **Unveiling the microbiome of *Haplaxius crudus:* the coconut lethal yellowing phytoplasma vector**
Puch-Hau, C., Lara-Pérez, L., Pérez-Garfias, B., Nic-Matos, G., Córdova-Lara, I., Oropeza-Salín, C., Oros-Ortega I., Sáenz Carbonell, L. *(page 50)*

16:30 – 17:00 **Coffee Break**

17:00 – 19:00 **Posters: Even numbers** (**see Index of posters on pages 282-284)**

19:00 – 20:00 **Keynote Lecture:**
Probing the universal energy-stress signaling network.
Du, H., Wu, Y., Shi, L., Ramon, M., Ye, R., McCormack, M., Rolland, F., Sheen, J. *(page 4)*
Moderator: Alfredo Herrera.

THURSDAY OCTOBER 31

PLANT STRESS RESPONSES: COMMON CHALLENGES FOR THE USA AND MEXICO

Moderator: Patricia Coello

09:00 – 09:30 **Plant environment interactions: the redox connection and autophagy involvement.**
Lascano, R. *(page 19)*

09:30 – 10:00 **Participation of SnRK1 kinases in plant energy signaling.**
Coello, P., Ruiz-Gayosso, A., Trejo, R., Porcel, A., Ávila, A. *(page 20)*

10:00 – 10:30 **Capsicum-geminivirus interaction: Comparison of two resistance mechanisms.**
Pablo-Rodríguez, J.L., Trejo-Saavedra, D.L., Rodríguez-González, M.J., Bárcenas-Rodriguez, R., Gongora-Castilla, E., Rivera-Bustamante, R. F. *(page 21)*

10:30 – 11:00 **Coffee Break**

11:00 – 11:30 **How interorganellar communication regulates plant stress responses.**
Dehesh, D. *(page 22)*

11:30 – 12:00 **Rare-earth elements and their role in the battle between plants and *Botrytis cinerea.***
Serrano, M. *(page 23)*

12:00 – 12:30 **Development of plant intrinsically disordered proteins-based fluorescence biosensors to track the osmotic environment.**
Cuevas-Velazquez, C. *(page 24)*

12:30 – 14:30 **Lunch**

CONCURRENT SESSIONS

MINISIMPOSIUM 5: STRESS RESPONSES AND CLIMATE CHANGE
Moderator: Gladys Cassab

14:30 – 14:50 **Carbon metabolism in bean pods during seed development.**
Bernal, L., Belmont, R., Martínez, V., Padilla-Chacón, D., Martínez-Barajas, J. E. *(page 51)*

14:50 – 15:10 **Analysis of the hydrotropic root response and elongation of mesocotyl in DTMA (Drought Tolerant Maize for Africa) hybrids under low water potential gradient conditions.**
Saenz, M.N., Nieto-Sotelo, J., Cassab, G.I. *(page 52)*

15:10 – 15:30 **Molecular and ecophysiological characterization of abiotic stress responses in *Marchantia polymorpha*.**
Flores-Martínez, D., Hummel, M., Oltehua-López, O., Arteaga-Vázquez, M., Bailey-Serres, J., Dorantes-Acosta, A. *(page 53)*

15:30 – 15:50 **Analysis of genetic variation associated to hydrotropism and deep planting resistance in maize (*Zea mays* L.) revealed genes controlling response to drought, growth, development and adaptation to global heating.**
Cassab, G.I., Sáenz-Rodríguez, M., Luján, R., Martínez-Guadarrama, J., Nieto-Sotelo, J., Eapen, D., Lledías, F., Puente-Báez, C., Campos-Torres, M.E. *(page 54)*

15:50 – 16:10 **Chromate treatment of *MEDIATOR* mutants triggers the splitting of the root meristem in *Arabidopsis*.**
López-Bucio, J., Ruiz-Aguilar, B., Raya-González, J. *(page 55)*

16:10 – 16:30 **Metabolic response to larval herbivory in *Physalis sp*.**
Trujillo-Pahua V., Vargas-Ponce, O., Rodriguez-Zaragoza, F., Ramírez-Romero, R., Montero-Vargas, J., Winkler, R., Sánchez-Hernández, C. *(page 56)*

MINISIMPOSIUM 6: EPIGENETICS
Moderator: Jose Luis Reyes

14:30 – 14:50 **The *Arabidopsis* methylome is robust to dark-induced senescence**
Trejo-Arellano, M.S., Mehdi, S., de Jonge, J., Dvorák Tomastíková, E., Köhler, C., Hennig, L. *(page 57)*

14:50 – 15:10 **The epigenetic behind the albinism in Agave**
De la Peña, C., Duarte-Aké, F., Us-Camas, R. *(page 58)*

15:10 – 15:30 **RNA methylation or the epi-transcriptomic control of gene expression in the moss *Physcomitrella patens***
Garcias, D., Jerez A., Covarrubias, A.A., Reyes, J.L. *(page 59)*

15:30 – 15:50 **Female gametogenesis, a pathway mediated by *MIR822* regulates germline linage in *Arabidopsis thaliana***
Durán-Figueroa, N. *(page 60)*

15:50 – 16:10 **Biotechnological implications of the analysis of plant meiotic mutants.**
Ronceret, A. *(page 61)*

16:10 – 16:30 **ptxD/Phi as a dominant and stable selectable marker system for cyanobacteria and microalgae**
González-Morales, S.I., Pacheco-Gutiérrez, N.B., Brito-Bello, A.A., Herrera-Estrella, L. R., López-Arredondo, D.L. *(page 62)*

16:30 – 17:00 **Coffee Break**

17:00 – 17:45 **Business Meeting**

17:45 – 18:45 **Keynote lecture:**

The relevance of carbon distribution towards reproductive organs in the resistance to terminal drought of common bean cultivars.

Covarrubias, A. A., González-Lemes, I., Acosta-Maspons, A., Cetz, J., Herrera-Estrella, A., Polania, J. A., Acosta-Gallegos, J. *(page 5)*

Moderator: Teresa Hernandez

18:45 –19:00 **Closure & Awards**

21:00 – 23:59 **Gala Dinner**

KEYNOTE LECTURES

Specification and differentiation of meiotic and somatic cells in maize anthers

Walbot, V.

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The old model for Angiosperm anther cell fate specification was modeled on ferns: as the four-lobed anther was generated, a large hypodermal cell in each lobe divided asymmetrically to form the subepidermal soma and a pre-meiotic archesporial cell (AR). Confocal microscopy of developing maize anthers disproved this model. Inspired by microarray results that highlighted metabolic accommodation to hypoxia, we demonstrated experimentally that low oxygen triggers AR cell fate. AR cells program neighbors to be soma by secreting a small protein MAC1 (MULTIPLE ARCHESPORIAL CELLS 1); MAC1 perception by an LRR-receptor (encoded by *ZmMsp1*, mutant *ems63089*) causes differentiation as primary parietal cells, which then divide periclinally to establish the subepidermal endothecium and internal secondary parietal cells (which later divide periclinally to establish the middle layer and tapetum, the final cell types of the lobe). Using mutants defective in cell fate acquisition or differentiation and single cell RNA-seq we have elucidated key steps in tapetal and AR development. AR differentiate into Pollen Mother Cells (PMC), which synthesize DNA in preparation for meiosis, and then there is “synchronous” meiosis in each lobe. Tapetal cells sequentially secrete beta-glucanase to remodel callose surrounding PMC, generate 24-nt phasiRNAs, accumulate exine components, reorganize as secretory cells, become binucleate, enzymatically release the microspores when meiosis is completed, and provide exine and other components to the microgametophytes. Open questions in our research: What roles do 24-nt phasiRNAs play? Why are they essential at normal corn growing temperature but not in cool conditions? How do 24-nt phasiRNAs move from tapetal cells to the PMC and/or meiocytes despite the presence of a thick callose coat? Projects are supported by the National Science Foundation in collaboration with Blake Meyers, Donald Danforth Center.

The integration of transcription factor and hormone signalling functions during gynoecium development

De Folter, S.

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Most angiosperms produce flowers with a gynoecium in their center, which is the female reproductive part of the flower. The initiation of the gynoecium lays the foundation for correct fruit development very early on. Transcription factors that promote meristematic activity have important roles in these early events. Furthermore, also hormonal signaling pathways, like cytokinin and auxin, are crucial for the correct development.

We want to understand how hormonal pathways such as cytokinin are integrated into local transcriptional networks and control specific organs like the gynoecium. We found that cytokinin signalling, which can provide meristematic properties required for Carpel Margin Meristem (CMM) activity and growth, is enabled by the transcription factor SPATULA (SPT) in the medial domain of the ovary. Preceding, the floral meristem must terminate to obtain carpel initiation. Besides known genes identified for floral meristem termination, we hypothesize that there are cytokinin-related targets of AGAMOUS (AG) that play a role in this process. We observed that altering cytokinin levels in the AG domain affects gynoecium development. At the moment, we are focusing on the determination of the spatio-temporal localization pattern of AG targets related with cytokinin signalling in early stages of flower development. Furthermore, we are studying which parts of the cytokinin biosynthesis pathway are regulated by the transcription factor SHOOT MERISTEMLESS (STM) during gynoecium development. Moreover, we generated protein-protein interaction data for gynoecium-expressed transcription factors. All together, we are obtaining a deeper understanding of the molecular mechanisms and the regulatory networks controlling gynoecium development.

Probing the universal energy-stress signaling network

Du, H., Wu, Y., Shi, L., Ramon, M., Ye, R., McCormack, M., Rolland, F., Sheen, J.

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The integration of energy-stress signaling network to promote fitness, adaptation, survival and longevity is remarkably conserved from single-cell eukaryotes to multicellular plants and humans. The yeast SNF1, plant SNF1-related protein kinase1 (SnRK1) and human AMPK are evolutionarily conserved master integrators orchestrating critical energy homeostasis between growth and stress responses and modulating various metabolic pathways and developmental transitions. Despite striking structural and functional conservation of the heterotrimeric energy sensor protein kinases, how the protein kinase complexes sense cellular energy status and reprogram transcriptome and diverse metabolic functions triggered by seemingly unrelated stress stimuli remain enigmatic in plants. In the past decade, gene redundancy and embryonic lethality have hampered genetic and molecular analyses of the plant energy sensor kinase functions. We have developed innovative chemical genetic tools and strategies to unravel the sensing and signaling mechanisms underlying the universal energy-stress regulatory network in plants. As global climate changes have led to increased incident of extreme weather and various environmental stresses that profoundly impacted plant-based ecosystems and agriculture, elucidating the convergent energy-stress signaling network may provide new targets and strategies for engineering broad stress tolerance in plants.

The relevance of carbon distribution towards reproductive organs in the resistance to terminal drought of common bean cultivars

Covarrubias, A. A.*[1], González-Lemes, I.[1], Acosta-Maspons, A.[1], Cetz, J.[2], Herrera-Estrella, A.[2], Polania, J. A.[1], Acosta-Gallegos, J.[3]

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[3] *Campo Experimental Celaya, Instituto Nacional de Investigación Forestal, Agrícola y Pecuaria (INIFAP), Celaya, México*

Common bean (*Phaseolus vulgaris* L.) is one of the most consumed legumes in the human diet. However, a major problem for this rainfed crop is the decrease in grain yield caused by terminal drought (TD). Despite its importance as a good source of proteins in Latin American and African countries, TD remains a threat to common bean farming, with losses reaching more than 80% in the last few years [Polania *et al.*, Euphytica 210: 17, 2016].

The aim of this research is to investigate the impact of TD on carbon distribution from leaves to pods and seeds and, its relationship and relevance to grain yield in common bean cultivars with different TD resistance. Previous reports and data obtained in our laboratory, and others, indicate that common bean cultivars considered resistant to TD are more efficient in remobilization of carbon reserves for seed filling under TD compared to TD sensitive cultivars, suggesting that carbon distribution is modulated by water deficit conditions [Polania *et al.*, Euphytica 210: 17, 2016; Cuéllar-Ortiz *et al*., Plant Cell Environ 3: 1399, 2008; Rosales-Villegas *et al*., Plant Physiol Biochem 56: 24, 2012].

In this work, we present physiological and molecular data supporting the relevance of photosynthate distribution to pods and seed in TD sensitive and resistant common bean cultivars. We have also conducted TD sugar accumulation and transcriptomic experiments of source leaves, pods and seeds of these cultivars. The molecular analyses have allowed the identification of transcripts/genes responding to TD stress conditions in those organs that suffer a major impact and are critical to final yield at this stage. Altogether, these findings exhibit the metabolic mechanisms allowing bean plants to withstand TD and be able to successfully achieve grain production under these adverse conditions.

Plenary Lectures

Tuesday, October 29

Improving Photosynthetic Efficiency for Improved Crop Yield

South, P., Cavanagh, A., Liu, H., Ort, D.

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Feeding the world's current population already requires 15% of the total net primary productivity of the globe's land area and that will need to increase to 25% in order to meet the projected increase in agricultural demand this century. This near doubling of food production will have to be accomplished on globally declining acreage and during a time in which there will be ever increasing demand on cultivated lands for the production of bioenergy crops, while in the face of a changing global environment that has already resulted in decreasing global yield of some of the world's most important food crops. The yield potential of crops is determined by their efficiency of capturing available light energy (ε_i), the efficiency of converting intercepted light into biomass (ε_c), and the proportion of biomass partitioned into grain (η). The remarkable yield gains of the Green Revolution in the middle of the 20th century resulted from plant breeders bringing η and ε_i for major crops close to their theoretical maxima, leaving improved photosynthetic efficiency as the only yield potential determinant with sufficient capacity to double crop productivity. Opportunities to improve photosynthetic efficiency exist in readapting photosynthesis to the rapid changes in atmospheric composition and temperature, in redesigning photosynthesis for agricultural production and in applying synthetic biology to bypass evolutionary limitations and inefficiencies in photosynthesis. Recent work using a synthetic biology approach to lower the energetic cost of photorespiration will be presented.

Unlocking the potential of *Agave* species as sustainable crops for marginal lands under a changing climate

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Despite the economic importance of agaves as the providers of the raw material for the tequila and mezcal industries for over 200 years, no formal breeding programs have ever been established for these species. Several factors have contributed to this situation including the strict regulation imposed by the CRT, the prolonged life cycle and perennial monocarpic nature of the plants, their unique anatomy and the exclusive practice of asexual propagation. However, agaves are perfectly adapted to survive under extreme environmental conditions and there is a growing interest for the exploitation of these species as sustainable and economically viable crops for marginal, mainly arid terrain. Our research is focused on developing the tools necessary to analyze agaves at the molecular and genetic levels and to facilitate the implementation of breeding and selection in *Agave* species. To this end we have developed transcriptome-based strategies to identify and characterize genes associated with agronomically important traits such as the vegetative-to-reproductive transition and carbohydrate metabolism. A candidate gene approach has allowed us to isolate cDNAs encoding the enzymes involved in fructan metabolism and for the floral integrator proteins of the Flowering locus T family. Additionally we have developed an *A. tumefasciens* based transformation protocol and in collaboration with researchers from the “Centro de Investigaciones en Óptica”, a method to measure succulence based on Terahertz imaging.

PhasiRNAs as modulators of traits in plants

Meyers, B.

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In plants, 21 or 22-nt miRNAs or siRNAs typically negatively regulate target genes through mRNA cleavage or translational inhibition. Heterochromatic or Pol IV are 24-nt and function to maintain heterochromatin and silence transposons. Phased “secondary” siRNAs (phasiRNAs) are generated from mRNAs targeted by a typically 22-nt “trigger” miRNA, and are produced as either 21- or 24-mers via distinct pathways. Our prior work in maize and rice demonstrated the temporal and spatial distribution of two sets of “reproductive phasiRNAs”, which are extraordinarily enriched in the male germline of the grasses. These two sets are the 21-nt (pre-meiotic) and 24-nt (meiotic) siRNAs. Both classes are produced from long, non-coding RNAs, generated by hundreds to thousands of loci, depending on the species. These phased siRNAs show striking similarity to mammalian piRNAs in terms of their abundance, distribution, distinctive staging, and timing of accumulation, but they have independent evolutionary origins. The functions for these small RNAs in plants remain poorly characterized. In monocots, the 24-nt phasiRNA pathway, triggered by miR2275 and abundant during meiosis, requires a recently-diverged Dicer known as DCL5, an interesting evolutionary elaboration of this pathway. I will describe our recent work investigating the functions of plant phasiRNAs, their diversification across crop plants, and their roles in modulating traits of agronomic importance in plants, including male fertility.

Topological Data Analysis (TDA) as a method to comprehensively measure the plant form

Chitwood, D.

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Embedded in all shapes is information. For all lifeforms, morphology—at the cellular, tissue, organ, and organismal levels—arises from genetic and environmental influences, and their interaction. These influences on the organismal form can be statistically determined, and mathematical models derived to predict which forms will arise under certain genetic and environmental scenarios. The diversity of plant forms that have arisen through evolution, and the domesticated morphologies of plants that have been artificially selected to create the major crops of the world, have benefited humanity incalculably, and directly result from alterations to plant architecture. Despite morphology being intrinsically linked to the sizes of grains and fruits, the yields of branching inflorescences, the canopies of fields, and the economics of root architectures, and even with intense interest in developing sophisticated genetic technologies and statistics to model these traits, we lack methods to adequately quantify plant morphology comprehensively and across scales. 1) Technologies that can comprehensively measure plant morphology in 3D at high resolution remain underused, 2) the real “shape” of plants that is dynamic and 4D, revealing itself through time, has almost never been captured, and 3) even if 3D and 4D datasets of plant morphology are produced, a mathematical framework to quantify the data is lacking.In this talk, I will discuss the use of X-ray Computed Tomography (CT) to create 3D models of plants and time-lapses of plant growth. A mathematical method that measures shapes comprehensively in any number of dimensions, Topological Data Analysis (TDA), will be used as a universal framework to analyze the resulting volumetric image data from any plant species and for any application.

Genetic diversity in world's largest wheat collection may hold the key to food security

Sansaloni, C.[1]; Franco, J.[2]; Santos, B.[3]; Singh, S.[9]; Petroli, C.[1]; Campos, J.[1]; Dreher, K.[1]; Payne, T.[1]; Marshall, D.[4]; Kilian, B.[5]; Milne, I.[4]; Raubach, S.[4]; Shaw, P.[4]; Stephen, G.[4]; Carling, J.[6]; Saint Pierre, C.[1]; Burgueño, J.[1]; Crosa, J.[1]; Kehel, Z.[6]; Amri, A.[6]; Kilian, A. [7]; Wenzl, P.[8]; Caccamo, M.[3]; Pixley, K.[1]

[1]CIMMYT- International Wheat and Maize Improvement Center, Mexico; [2]Universidad de la República del Uruguay, Uruguay; [3]NIAB- National Institute of Agricultural Botany, UK; [4]James Hutton Institute, UK; [5]The Crop Trust, Germany; [6]Diversity Arrays Technology, Australia; [7]CIAT- International Center for Tropical Agriculture; [8]ICARDA- International Center for Agricultural Research in the Dry Areas; Geneshifters, Pullman, Washington, USA 99163

Genetic diversity is the base for any breeding program in agriculture. In wheat, undomesticated wild species, crop wild relatives (CWR), and landraces represent sources of new variation for this process. However, their resilience and adaptive capacity mechanisms remain largely untapped and poorly understood. Seed of Discovery project (https://seedsofdiscovery.org) has genotyped, in a large percentage, the biggest wheat germplasm bank in the world, which is located at CIMMYT. Here, we evaluated 80,000 accessions belonging to CIMMYT and ICARDA germplasm banks; this represents the largest crop diversity analysis ever conducted on any species using DArTseqTM technology. The analysis was divided into three biological categories: 56,342 domesticated hexaploid, 18,946 domesticated tetraploid, and 3,903 CWR. Our analysis has identified more than 300,000 filtered high-quality SNPs and SilicoDArT markers. The generated markers were aligned to three reference maps: the IWGSC RefSeq v1.0 reference genome, the durum wheat genome (cv. Svevo), and the DArT consensus map. On average, 72% of the markers aligned uniquely on the reference genomes and 50% are linked to genes. The diversity analysis revealed landraces that host unexplored allele variants, and these could be incorporated into the wheat breeding programs. Heretofore, this represents a unique resource for the wheat scientific community, to explore new alternatives and develop the crops of the future. We believe that this new information is a fantastic option to use as a tool to face the pressure caused by climate change and gives us insight into the importance of maintaining genetic diversity in crops.

A new model of root development revealed by CRISPR and Single-Cell RNA Seq

Ortiz-Ramírez, C.[1], Demesa-Arevalo, E.[2], Xu Xiosa[2], Gingeras, T.[2], Jackson, D.[2], Gallagher, K.[3] and Birnbaum, K.[1]

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Tissue patterning is a fundamental aspect of development that underlies morphological diversity giving rise to important productive traits. However, the genetic mechanisms driving the organization of cell types into specific tissues is poorly understood in plants. To pinpoint the precise spatial and temporal changes in gene expression that lead to pattern formation, a high resolution is needed in transcriptional profiling. We used the Maize root as a biological model and Single-Cell RNA sequencing as an experimental tool to identify mechanisms controlling tissue patterning. By profiling thousands of cells, we created the first single-cell atlas of the maize root. This database allowed us to describe novel expression patterns and protein movement dynamics of SHORT-ROOT and SCARECROW, two important developmental regulators. Functional characterization of CRISPR mutants showed they control patterning in novel ways by regulating stele size and the number of cortex cell layers. Based on these results, we propose a new model for root radial patterning that helps explain morphological variation observed across species.

Wednesday, October 30

How leaves grow wide: *NARROWSHEATH1* controls mediolateral outgrowth of lateral organs.

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The mechanisms whereby lateral organ initial cells are organized from the peripheral zone of the shoot apical meristem (SAM) are poorly understood. The maize gene *NARROWSHEATH1* (*NS1*)/ *WOX3* is expressed at the marginal boundary of leaf founder cells in the SAM and in young leaf primordia, where it mediates mediolateral outgrowth. To investigate the mechanisms of NS1 function, we used ChIP-seq of NS1 followed by laser-microdissection RNAseq of *ns* mutant and wild type primordial margins to identify gene targets that are bound and modulated by NS1. In a comparative approach, ChIP-seq was also performed on the Arabidopsis WOX3 paralog PRESSED FLOWER1 (PRS1), to identify conserved mechanisms of founder cell recruitment and primordial outgrowth in maize and Arabidopsis. These data, combined with microscopic analyses of cell division dynamics, reverse genetic analyses of homologous NS1/WOX3 target genes in Arabidopsis, and *NS1* overexpressing plants in maize suggest that NS1/WOX3 controls mediolateral outgrowth by direct repression of growth inhibitory genes, and indirect promotion of cell division in primordial leaf margins. Intriguingly, the homologous *WOX* genes *WUS1* and *WOX5* are expressed in the organizing centers of the Arabidopsis SAM and root meristem respectively, whereupon these protein products traffic to adjoining cells to activate stem cell identity non-autonomously. In contrast, our previous data revealed that PRS1/WOX3 does not traffic, and these latest data suggest that NS1/*WOX3* stimulates primordial cell division in the same margin initial cells where it is transcribed.

IRK, a transmembrane receptor kinase, is polarly localized and represses root ground tissue cell divisions

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The nearly invariant organization of cells and tissues in the Arabidopsis root is maintained by stringent control of the timing and orientation of cell division. Cell polarity and directional signaling are frequently proposed to have a key role in these processes; yet, beyond transport proteins, very few proteins with polar localization have been characterized. We have identified a set of transmembrane receptor kinases, POLARLY LOCALIZED KINASEs (PLKs) that accumulate to specific plasma membrane domains in various root cell types. Here, we describe one PLK named IRK (INFLORESCENCE AND ROOT APICES RECEPTOR KINASE) that is polarly localized in and required for patterning of the root ground tissue. *irk* roots have ectopic endodermal cell divisions in the radial axis leading to more than eight endodermal cells per ring of cells and early middle cortex formation. We find that the IRK-GFP fusion protein is polarly localized to the outer plasma membrane domain of endodermal cells and that IRK-GFP localization varies in distinct cell types. Differences in IRK-GFP localization in the ground tissue coincide with cell division defects we observe in *irk*, suggesting that IRK localization to different regions of the plasma membrane is functionally important. We propose that IRK functions in a directional signaling pathway that inhibits specifically oriented cell divisions, which, ultimately, restricts ground tissue proliferation in the root's radial axis.

Leveraging Phenomic Approaches to Accelerate the Development of More Resilient Crops

Lorence, A.

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L-Ascorbic acid (AsA, vitamin C) is the most abundant water-soluble antioxidant found in plants. Ascorbate has a wide variety of physiological roles including being an enzyme cofactor, a scavenger of free radicals, and donor/acceptor of electrons in the chloroplast. Ascorbate protects plant tissues against damage caused by reactive oxygen species produced through normal oxygenic metabolism or generated from biotic and abiotic stresses. In plants ascorbate is biosynthesized via four different routes that utilize L-galactose, L-gulose, *myo*-inositol and galacturonate as main precursors. Understanding the regulation of the ascorbate metabolic network and leveraging this knowledge to accelerate the development of more resilient crops is a primary focus in the Lorence Laboratory. In this presentation we will describe how by combining transcriptomics and phenomics approaches we are gaining insights about the mechanisms mediating the increased growth rate, biomass accumulation and enhanced abiotic stress tolerance we have observed in plants over-expressing enzymes involved in the conversion of *myo*-inositol into ascorbate.

Dr. Lorence co-leads the Wheat and Rice Center for Heat Resilience (WRCHR; http://wrchr.org/), a consortium of Nebraska-, Kansas- and Arkansas-based researchers looking to find novel sources of tolerance to high night temperature stress in rice and wheat, the two crops that provide the most calories to people worldwide. This environmental challenge is a key factor negatively affecting the quality of the grain and yield in rice. We are leveraging natural variants that are part of a rice diversity panel to identify novel sources of high night temperature resilience in field scale experiments. In this presentation we will highlight the progress achieved during the first field season of this ambitious project in Arkansas.

DICER-mediated Reprogramming of Cell Fate Specification in *Marchantia polymorpha*

Aguilar-Cruz, A.[1], Dorantes-Acosta, A.[1], Oltehua-López, O.[1], Kohchi, T.[3], Ishizaki, K.[4], Bowman, J.[5], Grimanelli, D.[6], Haseloff, J.[2], Arteaga-Vázquez, M.[1]

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Small non-coding RNAs (sRNAs) are essential regulators of gene expression in eukaryotes at both the transcriptional and post-transcriptional level. *DICER* genes are involved in the biogenesis of the vast majority of sRNAs including microRNAs (miRNAs) and small interfering RNAs (siRNAs). Here we report on the characterization of mutant alleles of the *Marchantia polymorpha DICER-LIKE 1* gene (Mp*DCL1*) that we obtained through genome editing using the CRISPR-Cas9 system. In addition to a dramatic delay in development, vegetative propagules (gemmae) of Mp*dcl1* mutants, exhibit additional apical notches and contain ectopic mucilage papillae. At the reproductive level, Mp*dcl1* mutants display feminized antheridia and abnormal archegonia with supernumerary egg cells. Strikingly, we also observed the direct reprogramming (*i.e.* without the formation of thalli or gemma cups) of single epidermal cells into ectopic gemmae. *MpDCL1* is expressed through *M. polymopha* life cycle from the first layer of cells derived from gemmae initials, expanding to the midrib and in both gametophores. Taken together, our results show that Mp*DCL1* plays a central role in plant development through the regulation of cell specification of structures derived from single epidermal cells that expand out from the epidermal surface during the formation of vegetative propagules and restricts the formation of female gametes. This indicates Mp*DCL1* is an essential gene required for both asexual and sexual reproduction in *M. polymorpha*.

An unexpected role of trehalose phosphate phosphatases in meristem determinacy.

Claeys, H., Vi, S.L., Xu, X., Satoh-Nagasawa N., Eveland, A.L., Goldshmidt, A., Feil, R., Beggs, G.A., Sakai, H., Brennan, R.G., Lunn, J.E., and Jackson, D.

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Plant growth depends upon meristems, pools of stem cells that are maintained throughout plant life. Meristem identity is crucial for plant development, and has been selected during crop domestication to enhance yields. Trehalose-6-phosphate (T6P) phosphatases (TPPs) control meristem determinacy, but their mechanism of action remains mysterious. T6P is an important signal controlling energy balance, flowering time and stress responses in plants, yet its level is very low, suggesting a signaling rather than metabolic role. In a screen for enhancers of the maize *tpp* mutant *ramosa3* (*ra3*), which has more inflorescence branches due to reduced meristem determinacy, we identified several alleles of *TPP4*, a redundant paralog. Besides its role in inflorescence architecture, *TPP4* also controls flowering time. Unexpectedly, analysis of an allelic series found no correlation between TPP4 enzymatic activity and branching, suggesting it functions through a non-enzymatic or moonlighting activity. Accordingly, a catalytically inactive version of *RA3* complements the *ra3* mutant. Moreover, RA3 and TPP4 both localize to the nucleus. Mutating *TPP12*, another *RA3* paralog, did not enhance *ra3*, suggesting a functional divergence between canonical TPPs involved in carbon homeostasis, and moonlighting TPPs that regulate development independent of their enzymatic activity. We will discuss these findings, as well as recent results that RA3 interacts with nuclear proteins that may explain its non-conventional mechanism of action.

Genetic basis of domesticated traits in date palm (*Phoenix dactylifera*)

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Date palms (*Phoenix dactylifera*) are an important fruit crop of arid regions of the Middle East and North Africa, but is also a crop in California, both in Mexico and the USA. Date palms are perennial plants with long life cycles, which makes difficult to implement short-term plant breeding strategies. In this talk, I will give some insights on what we have learned about the possible origins of date palm, selective sweeps identifying candidate loci probably underlying date palm domestication, and genome-wide association mapping to detect loci responsible for sex determination and fruit traits. Among these results I will highlight the findings on a R2R3 myb-like orthologue of the oil palm *VIRESCENS*, which controls variation in fruit color in date palm cultivars. I will also highlight loci associated to sugar composition. This work shows the relevance of developing genomic resources in non-model perennial plants to identify the genetic basis of natural variation, and plant domestication.

Thurthday, October 31

Plant environment interactions: the redox connection and autophagy involvement.

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The life, as open thermodynamic system, depends on the continuous energy and fluxes, accomplished through redox transformation reactions, such as photosynthesis and respiration. The aerobic living forms supposed a great adaptive advantage, however brought about the generation of toxic compounds. The reactive oxygen species (ROS), common by-products of aerobic metabolism which generation markedly increase under different stress conditions, are highly toxic compound reacting with cellular macromolecules and inducing aging, natural and stress-induced senescence phenomena. These features constitute the dark side of ROS. However, since more than a decade ago, this paradigm has changed, and nowadays ROS and the cellular redox state, determined by the subcellular ROS generation/degradation ratio, the reduction of chloroplast and mitochondria electron chains, the levels of reducing equivalents and the level and sugars flux¸ are recognized as local and systemic signals that modulates growth, development, stress and acclimation responses, symbiotic and pathogenic microorganism interactions and programed cell death processes. Our research group has been accompanying the ROS paradigm change, involving biophysical, molecular, biochemical, cellular, physiological and eco physiological approaches. Nowadays, using crop plants like Glycine max (Gm) and model plants like Arabidopsis thaliana and Physcomitrella pattens, we are studying the relationship among redox changes, primary metabolism, regulation of autophagy and senescence under different stress conditions. We have demonstrated involvement of redox changes and autophagic genes during the Gm-Bradyrhizobium japonicum (Bj) interaction under control and stress conditions, where the tip growth processes and root hair cell death are key regulatory events of nodulation. Likewise, during Gm-Bj interaction, we have also characterized a transient systemic redox change, microRNAs expression pattern changes and its association with induced systemic responses and increase tolerance to photooxidative stress tolerance and resistance to Soybean Mosaic Virus infection. The systemic redox change is dependent on Nod factor receptor and the ISR-like response on the Autoregulation of Nodulation. Likewise, we are studying the redox regulation of autophagy under different stress conditions.

Participation of SnRK1 kinases in plant energy signaling

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Plants are sessile organisms frequently exposed to growth conditions that reduce the availability of nutrients and leads to energy stress. Recent developments in plant cell signaling have emphasized an important connection between metabolic regulation and stress signaling, helping plants to resist and adjust to environmental changes. Multiple pieces of evidence indicated that kinases of the SnRK1 family have a significant role in restoring energy homeostasis. SnRK1 perform this function through the direct regulation of enzymes that control metabolic processes and by promoting extensive changes in gene expression. The SnRK1 is a heterotrimeric complex consisting of an α catalytic subunit, and two regulatory β and γ subunits. In Arabidopsis, two of the regulatory β subunits (SnRK1 β1 and SnRK1 β2) and a plant-exclusive γ subunit (SnRK1βγ) contain a carbohydrate-binding module (CBM) at the N-terminus. Alternative approaches to determine the role of the CBM domain suggest its participation in kinase activity regulation. We observed that regulatory subunits bind maltose, a relevant product of starch degradation. The kinase activity of immunoprecipitated complexes containing the βγ regulatory subunit was positively regulated by maltose. Recombinant complexes with the SnRK1α1 catalytic subunit, SnRK1βγ and three different β subunits showed that maltose only affected on a complex formed with the β3 subunit. Truncation of the CBM domain form SnRK1 βγ abolished the maltose activation of the complex, and the activity was significantly reduced, indicating that the CBM is a positive regulator of SnRK1. A direct association between SnRK1 and starch metabolism has been established because *snrk1* mutants accumulate starch at the end of the night. In this talk, we will discuss how the SnRK1 contributes to plant responses to the energy stress-induced by nutrient limitation.

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Capsicum-geminivirus interaction: Comparison of two resistance mechanisms.

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Mexico is the second most important producer of peppers. Members of the Geminiviridae family infect a wide variety of crops, including members of the genera *Capsicum, Solanum, Nicotiana* and *Physalis*. Pepper golden mosaic virus (PepGMV) and Pepper huasteco yellow vein virus (PHYVV) are the most important viruses in Mexico for Capsicum crops. During our studies on Capsicum-geminivirus interactions, we have encountered two different mechanisms of resistance.

Recovery. In some geminivirus-host interactions, infected plants show recovery, a phenomenon characterized by symptoms disappearance in newly emerging leaves. In pepper-PepGMV interaction, the recovery process involves a silencing mechanism that includes both post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS) pathways. More recently, we have investigated if other defense pathways are also involved in the recovery process. Pathways of special interest are the ones involved in jasmonic acid and ethylene metabolisms. Several genes were studied (expression patterns and *virus-induced gene silencing*, VIGS) to verify their involvement on the infection cycle. Silencing of selected genes (i.e. CaLOX2, CaJAR1) dramatically affect the recovery of the plant suggesting an importat role.

Habanero Resistance. In a wide screening of *Capsicum* accessions to identify resistance to geminivirus infection, accession BG-3821 of *Capsicum chinense* Jacq. (collected in Yucatan) was identified. Studies with protoplasts and GFP suggested that both replication and movement processes were impaired in the resistant habanero accession. Expression analysis of selected genes suggested that the resistant line was able to respond faster than the susceptible line after infection with PepGMV. More recently, we used a transcriptomic approach to compare several profiles from R and S lines (segregated from the originally field-identified plant). RNA samples from resistant and susceptible plants were collected at 0, 2, 4, 8, 12, 24, 48 and 96 hrs after PepGMV inoculation. cDNA libraries were prepared and sequenced (Ilumina). Results of the transcriptomic analysis will be presented. Our findings support the idea of a faster response by the resistant line. However, the results of the comparison at 0 Hr, suggest that a non inducible defense mechanism against viral infections could be already present and collaborate in the overall response.

How interorganellar communication regulates plant stress responses

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Interorganellar communication is an evolutionary necessity for maintenance of cellular homoeostasis in response to prevailing environment that, in part, is exquisitely controlled via retrograde-signaling pathways. We have identified a novel stress-specific plastidial retrograde signalling metabolite, methylerythritol cyclodiphosphate (MEcPP), previously known solely as an intermediate in the isoprenoid biosynthetic pathway. The additional function of MEcPP as a stress sensor and a coordinator of transcriptional and post-transcriptional regulation of key stress-responsive nuclear genes, has unraveled the central role of this metabolite in cellular functions in response to a wide range of environmental and developmental cues. To identify the underlying molecular mechanism of the MEcPP-mediated stress responses, we have performed a multi-omics approach. These studies have led to the identification of a transcriptional hub activated by MEcPP, and have further established a previously unrecognized link between this plastidial retrograde signal and the transcriptional reprogramming of endoplasmic reticulum genes critical for readjustment of protein-folding capacity in stressed cells. Moreover, we have gained an insight into the molecular mechanism by which MEcPP alters subcellular structures and contributes to phytochemical diversity. In brief, we have advanced our understanding of how plastids through accumulation of MEcPP reprogram a repertoire of intricate networks crucial for coordinating the physiological and metabolic processes required for stress-induced developmental and adaptive responses.

Rare-earth elements and their role in the battle between plants and *Botrytis cinerea.*

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From all food produced for human consumption, every year 1.3 billion tonnes are lost. Only during post-harvest, 25% to 50% of the production can be lost due to plant diseases induced by microorganisms and by suboptimal handling and storage conditions. In particular, fungi of the genera *Alternaria, Aspergillus, Botrytis, Fusarium, Geotrichum, Gloeosporium, Penicillium, Mucor* and *Rhizopus* are responsible for most of these losses. In particular, the necrotrophic fungus *Botrytis cinerea,* has been classified a the second most important pathogen in the agriculture, since can affect more that 200 crops. Synthetic chemical fungicides have been used to control fungi-induced diseases. However, increase on worldwide regulatory policies and the demand to reduce their application, due to potential harmful side effects to the environment and to humans, have led to searching for new alternatives such as the biocontrols. In our research group, have isolated and characterized several biocontrols of *Botrytis cinerea*, including a rare-earth element. In this seminar, the results of the molecular characterization of this compound will be presented.

Development of plant intrinsically disordered proteins-based fluorescence biosensors to track the osmotic environment

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Plants constantly experience fluctuations in water availability due to changes in the osmotic potential of their extracellular environment. In contrast to bacteria, yeast, and animals, where the molecular identity of the osmosensory pathway is well characterized, little is known about this process in plants due to the lack of methods to visualize the immediate effects after osmotic stress is perceived. In order to better understand the mechanisms of osmotic stress sensing and acclimation in plants, I have designed a strategy to track osmotic changes *in vivo* with the use of genetically-encoded fluorescent biosensors. I chose Arabidopsis intrinsically disordered stress proteins as the sensory domain because of their high flexible nature and their reported environmentally-driven conformational changes. The biosensors developed, named Sensors Expressing Disordered domains (SED), can dynamically monitor changes in the osmotic environment in living bacteria, yeast, plant, and mammalian cells. Interestingly, SEDs exhibit a fast change in localization from the cytoplasm to discrete spherical bodies in response to the stress. Such bodies exhibit liquid-like properties. This unexpected behavior could be used as a second read-out of SEDs. Finally, I will present a strategy to investigate how the physicochemical environment (macromolecular crowding and osmotic potential) might regulate cellular functions by compartmentalization of particular proteins into dynamic membraneless structures.

The dynamic imaging of processes occurring immediately after the osmotic shock will contribute to the understanding of the biophysical effects of this stress in living systems and the dynamic downstream events that enable acclimation and survival of crops and other organisms.

Keywords: Osmosensing, Fluorescent Biosensors, Macromolecular crowding, Intrinsically Disordered Proteins, Plant Biology.

CONCURRENT SESSIONS &

MINISIMPOSIA

MINISIMPOSIUM 1: DEVELOPMENT AND ORGAN SPECIFICATION

Signals back and forth: the epistasis of plastid translation and the ApoCarotenoid Signal 1 (ACS1) in leaf development

Escobar-Tovar, L.[1], Sierra[1], J., Hernández-Muñoz[1], A., Mathioni[2], S., Cordoba[1], E., McQuinn[3], R., Meyers[2], B., Pogson[3], B., León[1], P.

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The characterization of the albino *chloroplast biogenesis 5* (*clb5)* mutant of *Arabidopsis thaliana*, lacking zeta-carotene desaturase (ZDS) function, has provided evidence that the accumulation of linear carotenoid intermediates are capable of modulating nuclear and plastid gene expression, chloroplast biogenesis and leaf morphology. Genetic and biochemical analyses demonstrated that the *clb5* finger-like leaf and transcriptional phenotypes were due to cleavage of phytofluene and/or zeta-carotene isomers to produce the ApoCarotenoid Signal 1 (ACS1). It has also been demonstrated that the CCD4 enzyme is important in the ACS1 biogenesis, since the *ccd4-clb5* double-mutant ameliorates leaf lamina and gene expression patterns. On the other hand, morphological and molecular evidences supported that defects in leaf development caused by the inhibition of plastid translation (IPT) are morphological and developmentally indistinguishable to those found in *clb5*. Expression analysis of *clb5* demonstrated that the accumulation of ACS1 affects the expression of diverse nuclear genes that encode essential plastid ribosomal proteins, and in consequence impairs plastid translation. Genetic analysis indicated that the inhibition of plastid translation is epistatic to ACS1 and is a direct cause of leaf morphological alterations present in the *clb5* mutant. Moreover, both responses require the participation of the GUN1 protein. It was also demonstrated that light is an important component for the ACS1-mediated signaling, acting upstream of IPT. Interestingly, our work supported the idea that the plastids present in the leaves of *clb5* and of plants impaired in plastid translation are physiologically different from the plastids present in other organs of these plants. Collectively, this work demonstrated that ACS1 and IPT, generated from specific conditions of plastid development, share a common signaling pathway. This research was supported by DGAPA-UNAM (PAPIIT IN204617) and CONACYT (CB 220534).

BOL modulates gynoecium development and its cytokinin response

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In contrast to other multicellular organisms, plants can make new organs post-embryonically. Plants contain groups of cells in an undifferentiated state, with active cell division, known as meristems. Cells on the meristem periphery obtain the capacity to develop into lateral organs. This meristematic activity is controlled by hormones and genetic regulators. The formation of new organs is related to the presence of the hormone auxin, observed in only one or a few cells just before organ primordium emergence, which are called the organ founder cells. BOL is an AP2/ERF transcription factor that functions at early stages of organogenesis and it has been proposed as a marker for the flower organ founder cells in Arabidopsis. An indirect connection between BOL and the auxin biosynthesis pathway has been reported. However, it has also been suggested that auxin function may be independent of BOL. Auxin and cytokinin are important for organ morphogenesis and the pathways of these two hormones are connected at different levels. The gynoecium is a key reproductive Arabidopsis floral organ. *drnl-2* (a BOL loss-of-function mutant) gynoecia show defects in the apical–basal axis, and these defects are similar to those observed when auxin signaling or biosynthesis is altered or when exogenous cytokinin is applied. Therefore, we characterized BOL expression during gynoecia development and found that, besides its founder cell activity, it also participates at later stages. Also, we studied the effects of its loss of function in the response to cytokinin treatment in order to explore its connection with the phytohormone cytokinin. We found BOL differentially modulates the response of the gynoecium to cytokinin at distinct stages, having possibly a dual role during gynoecium development. Now we are working towards clarifying the mechanism through which BOL modulates the cytokinin pathway and we are analyzing the relationship between BOL and cytokinin during vegetative development.

Uncovering the genetic regulation of determinate root growth in Cactaceae

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The primary root of many Cactaceae species exhibits determinate growth as a consequence of root apical meristem (RAM) exhaustion and differentiation of all root-apex cells soon after germination (Dubrovsky, 1997, Shishkova *et al.,* 2013). Determinate growth of primary and lateral roots leads to the formation of a compact root system that provides seedlings with an advantage for survival in arid and semiarid environments. To explore the genetic regulation of the RAM exhaustion, we sequenced the *Pachycereus pringlei* (Cactaceae) transcriptome and microtranscriptome of the primary-root apex, and the whole-root degradome. The root apex transcriptome was *de novo* assembled, transcript abundance during root development was assessed and the root-apex transcriptional regulatory network was inferred. For microRNA identification, we extracted the *P. pringlei* sequences flanking mapped smallRNA reads in the *de novo* assembled transcriptome (Rodriguez-Alonso *et al*., 2018) and published low coverage genome (Copetti *et al.*, 2017); and used them to predict the stem-loop structures of the putative microRNA precursors. Degradome-seq analysis, based on the precise site of the target transcript cleavage within a sequence complementary to a plant microRNA, revealed previously reported in other species and novel targets of conserved microRNAs, and also evidenced the cleavage activity of novel microRNAs. The inverse correlation of certain microRNAs and their targets, including novel miRNA-target modules, in the *P. pringlei* primary-root apex, suggests the involvement in the RAM exhaustion of post transcriptional regulation mediated by microRNAs. Furthermore, our results suggest conservation of transcriptional modules of the RAM maintenance among angiosperms and the involvement of the novel lineage-specific transcripts in Cactaceae root development in the RAM exhaustion. *Acknowledgements*: This work was partially funded by PAPIIT-UNAM IN201318, IN200818 and CONACyT-CB 240055 grants.

Genomics of cellulose and lignin biosynthesis in *Agave tequilana* Weber

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Cellulose and lignin shape plant cell walls and together are the most abundant biopolymers at earth. Cellulose, a polymer of glucose units linked in long chains, is found in primary and secondary cell walls. Lignin adds rigidity mostly to secondary cell walls, and this compound is a mix of different monolignols units that are formed in the phenylpropanoid pathway. Recently, there is a growing focus is on *A. tequilana* Weber for biofuel production, because its high levels of soluble sugar and relatively low amount of lignin. Here, orthologous genes involving with cellulose and lignin biosynthesis in *A. tequilana* were identified by a data mining approach. For this, RNAseq data of 8 different transcriptomes of *A. tequilana* Weber (J. Simpson lab, unpublished data) were used. Our analysis identified orthologous genes encoding to enzymes for each step during cellulose or lignin biosynthesis. Agave genes show high homology levels compared to those reported in *A. thaliana* and monocots as maize and rice. Transcriptomic profiles of genes of both metabolic pathways in primary or secondary cell wall-enriched tissues are according as expected for their roles in cell wall metabolism. Additionally, DNA sequence motifs and expression patterns analysis allow proposing a classification for the agave CESA proteins, which are key players in cellulose biosynthesis.

Differential expression and interaction profiles of maize MADS-box dimer evolutionary variants

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The complex regulatory network that controls floral development is still not completely understood, especially in monocots. MADS-box transcription factors are key homeotic genes specifying floral organ identity. Interactions between themselves and with other proteins form quartets, which are critical to DNA-binding. In most species, it is unclear the identity of quartets assemble. In grasses, B-class MADS-box proteins differ in their interaction profiles, and show variable homodimerization over the time. B-class proteins APETALA3 (AP3) and PISTILLATA (PI), which form the male organs, bind DNA as obligate heterodimers with one another, but interestingly, both homo and heterodimerization occur particularly in monocots. In maize, only ancestral PI can form both and has been shown that obligate heterodimerization was fixed during domestication. We are using the maize PI ortholog STERILETASSEL SILKY EAR1(STS1) and evolutionary variants of STS1 to determine the mechanistic basis of the differential dimerization. *sts1* mutant complemented with STS1 homo and heterodimer were analyzed at floral morphology level. Also, differential expression and protein complex were analyzed by RNAseq and CoIP. At early stage of development, variants show subtle phenotypic differences. Transcriptional profiles and quantitative proteomics show higher plasticity in the homodimer complemented line and suggest a specialization and more fine tuning regulation of STS1 through the time. In the CoIP, protein kinases were identified, and phosphorylation of STS1 was observed using an Anti-pSer antibody. Future experiments will reveal whether identified protein kinases can phosphorylate STS1 and whether changes in phosphorylated aminoacids are able to modify DNA binding. Overall, our results will reveal mechanisms of shifting protein-protein interactions to gene regulatory evolution, and might help to identify novel factors involved in male sterility in maize.

Lateral root formation in *Arabidopsis thaliana* starts from a single founder cell: new insights into initiation process

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It is established that lateral root primordium (LRP) in *Arabidiopsis* is initiated from three laterally adjacent founder cells (FCs) viewed in transversal plane while there are two FCs in each file in longitudinal plane. However, it has recently been demonstrated that developed LRPs are composed of the progeny of a greater number of files of FCs than considered before (Wangenheim *et al*, 2016, Curr Biol 26:439), suggesting that FCs continue to be recruited after the beginning of LRP initiation. To gain a deeper insight in LRP initiation we performed a clonal analysis of LRP formation using *35S-DS1-H2B:YFP; HS-Ac* seedlings subjected to heat shock (Kurup et al Plant J 42:444) and confirmed its deductions with time-lapse confocal laser scanning microscopy experiments. Using a “hunting” approach (that will be outlined) for double transgenic line *p35S::H2B-RFP pUBQ10::NPSN12-YFP* that permits visualization of nuclei and plasma membrane, we have established that LRP development starts from a single FC representing previously unrecognized Stage 0 of LRP formation. Using the *pGOLVEN6* reporter line as FC marker (Fernandez *et al*, 2015, JXB 17:5245) and “hunting” approach we confirmed that LRP initiation starts from a single cell and that after its specification, laterally adjacent pericycle cells become gradually and progressively recruited for LRP morphogenesis as FCs. Studies of *DR5* promoter activity and responses to pharmacological treatments with NPA suggest that auxin signaling and transport are necessary and sufficient for gradual recruitment of FCs. In summary, we established for the first time that a gradual FC recruitment takes place during LRP initiation and morphogenesis. The fact that early steps of floral primordium development are also accompanied with FC recruitment suggests that common mechanisms operate during lateral organ primordium formation in shoots and roots. We thank to DGAPA-UNAM (IN200818) and CONACyT (237430) for support.

MINISIMPOSIUM 2: SIGNAL TRANSDUCTION

Microbial endophytes change phytohormone levels for drought and plant pathogens tolerance

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Microbial endophytes, mainly bacteria and fungi, are microorganisms that live within plant tissues without causing apparent damage. Of interest are fungal endophytes (mycobiota) due to their functional classifications as protective agents, mycorrhizae, dormant saprobes and dormant pathogens. Our research group isolated fungal endophytes from different cotton varieties under drought conditions, identified them and inoculated them back to cotton to observe their effect as protective agents against drought and insect pests. Results indicate that, when used as endophytes, fungal entomopathogens *Beauveria bassiana* (*B. bassiana*) and *Paecilomyces lilacinum* (*P. lilacinum*) induced increase in yield and tolerance against cotton aphid (*Aphis gossypii*), lygus bug (*Lygus hesperus*) and green stink bug (*Nezara viridula*). In addition, studies done in parallel, indicated that defense related hormones in cultivated cotton were also found in inoculated plants, and phytohormone hormonal levels were differently affected by the presence of the endophytes, in both the absence and presence of herbivory. These results coincide with what has been published for another beneficial microorganisms-plant mutualisms. Therefore, our findings support induced systemic defense responses in the plant, as a mechanism underlying endophyte-mediated tolerance to herbivory in cotton, in addition to accumulation of abiotic stress responsive phytohormones as abscisic acid (ABA). Colonization of plants by entomopathogenic fungal endophytes is mainly facultative and negative effects on herbivores have been shown in different endophyte-plant systems, therefore similar effects are likely to be ubiquitous, but modulated by the specific plant-endophyte combination involved in the interaction.

Winter winner, energy dinner. H^+-ATPase activity regulation through MAP kinases during cold conditions

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Cold acclimation is necessary for plants to survive freezing temperatures and is attained when plants are pre-exposed to low non-freezing temperatures. Cold acclimation triggers several signaling events such as the activation of mitogen-activated protein kinase (MAPK) cascades. The plasma membrane (PM) H^+-ATPase is a key enzyme for the plant cell and has transcriptional, translational and post-translational mechanisms of regulation. An important target of freezing damage is the PM and different mechanisms are activated during cold acclimation to protect it such as the release of COR15 proteins. We used wild type (wt) plants and *Arabidopsis* mutants lacking MPK3, MPK4 or MPK6 enzymes that were acclimated (AC) to 4°C or non-acclimated (NA). AC plants showed an increased survival rate as to the NA plants, except in *mpk6* mutants. We have characterized the activity, the kinetic behavior and the amount of the H^+-ATPase in the different plants. The results showed that AC decreased the H^+ ATPase activity in wt and *mpk3* plants, but not in *mpk4* nor *mpk6* mutants. The kinetic constants obtained were not associated with the amount of enzyme in the wt or mutant plants. Preliminary analysis of the PM sphingolipids showed that acclimation decreased sphingolipid levels but at different extent in wt or mutant plants. Preliminary analysis of transcripts from H^+-ATPase isoforms and sphingolipid biosynthesis enzymes showed that acclimation decreased *AHA2* and *SBH1* (a sphingolipid hydroxylase) mRNAs only in wt and *mpk3* plants. These results indicate that MPK3/4/6 may participate in the H^+-ATPase activity regulation in the cold acclimation process, possibly by modulating the sphingolipid environment of the enzyme.
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Trichoderma asperellum protects tomato plants against fungal infections diseases through inhibition of Reactive Oxygen Species Production

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Trichoderma species are fungi widely employed as plant-growth-promoting agents and for biological control of plant diseases. It has been estimated that approximately 60% of the commercial biological agents are made from *Trichoderma* spp.
Trichoderma antagonism toward fungal pathogens and include mycoparasitism, antibiotic production and competition for nutrients. Moreover, *Trichoderma* also exerts an indirect control against pathogens through the induced systemic response (ISR) in plant cells that results in an enhanced defense.
Trichoderma produces large amounts of fungal spores and this characteristic makes it ideal for inoculum production under laboratory conditions. Several commercial and laboratory-made solid formulations for mass production of *Trichoderma* have been reported. In this study, we evaluated a solid kaolin-based formulation to promote the absorption/retention of *Trichoderma asperellum* in the substrate for growing tomato plants. Kaolin is an inexpensive clay that is chemically inert over a relatively wide pH range, and this has been used to carry and preserve fungi and bacteria with herbicide activities.
Our results demonstrated that the unique implementation of this solid formulation resulted in an increased growth of the tomato plants, both in roots and shoots after 40 days of its application, indicating an optimal retention and survival of *T. asperellum* in the solid matrix.
On the other hand, the plants that were pre-treated with *T. asperellum* and then challenged with two fungal pathogens, *Fusarium oxysporum* and *Botrytis cinerea*, showed lesser severe wilting and stunting symptoms than non-treated plants. The pre-treatment with *T. asperellum* formulation inhibited Reactive Oxygen Species (ROS) production in response to the pathogens in comparison to plants that were only challenged with both pathogens, suggesting that decrease in ROS levels contribute to the protective effects exerted by *T. asperellum* in tomato.

Cornichon sorting and regulation of GLR channels underlie pollen tube Ca^{2+} homeostasis

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Compared to animals, evolution of plant calcium (Ca^{2+}) physiology has led to a loss of proteins for influx and small ligand–operated control of cytosolic Ca^{2+}, leaving many Ca^{2+} mechanisms unaccounted for. Here, we show a mechanism for sorting and activation of glutamate receptor–like channels (GLRs) by CORNICHON HOMOLOG (CNIH) proteins. Single mutants of pollen expressed *Arabidopsis thaliana* GLRs (*At*GLRs) showed growth and Ca^{2+} flux phenotypes expected for plasma membrane Ca^{2+} channels. However, higher-order mutants of *At*GLR3.3 revealed phenotypes contradicting this assumption. These discrepancies could be explained by subcellular *At*GLR localization, and we explored the implication of *At*CNIHs in this sorting. We found that *At*GLRs interact with *At*CNIH pairs, yielding specific intracellular localizations. *At*CNIHs further trigger *At*GLR activity in mammalian cells without any ligand. These results reveal a regulatory mechanism underlying Ca^{2+} homeostasis by sorting and activation of *At*GLRs by *At*CNIHs.

Analysis of cell signaling in *Capsicum chinense* response to pathogens: a vision of molecular dynamics in plant defense

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Peppers are fruits that belong to the *Capsicum* genus and constitute important crops that position Mexico as a world leader in the export of peppers. However, its production is threatened by different pathogenic organisms such as fungus and oomycetes. The cell signaling of the pathogen-plant interaction was studied in *Capsicum chinense* suspensions cells versus a microbial consortium with predominance of the fungus, analyzing the levels of membrane phospholipid and the transcription levels of diacylglycerol-kinase gene. The results showed morphological and metabolic changes during interaction and high transcription levels of the phospholipid pathways gene. Likewise, the *Capsicum* cells were inoculated with *Pythium ultimum*, an oomycete, analyzing the synthesis of salicylic acid and the expression of genes related to pathogenesis (*PRs*), demonstrating that *P. ultimum* causes morphological damage, induces endogenous AS increase and increases expression levels of PR1 genes. To analyze the specificity of the response with respect to the virulence of the pathogen, the attenuation of *P. ultimum* was developed to determine the proteomic and oligogalacturonide defense profile. These results show how biotic stress induces changes in the signaling pathway of the host cell that are key to plant defense.

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Self-incompatibility: a way for mothers to prevent a progeny with low fitness

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In Solanaceae, self-incompatibility (SI) is controlled by the specificity determinants: the S-RNase (female) and the SLF (male). SI occurs if there is an *S*-allele specific match between both determinants in the pollen tube (PT). Although, modifier genes (MG) unlinked to the *S*-locus are also required for SI. These genes are: *HTB*, *120K*, *NaStEP*, *NaTrxh* and *NaSIPP*.

Two models explain the pollen rejection response. One proposes that a suit of SLFs recognizes nonself S-RNases, which are ubiquitylated and degraded via the proteasome 26S, leaving self S-RNase free to degrade RNA in the PT. The second model proposes that S-RNases are taken up by PTs and sorted to a vacuole. In an incompatible cross, this vacuole is broken down, releasing S-RNases to the cytosol. Vacuole breaks down would be triggered by the presence of HT-B, which is degraded in a compatible cross. Regardless, both models do not contemplate the participation of other MGs discovered so far.
We propose a new version of the compartmentalization model that includes these MG. We suggest that NaStEP, a Kunitz-type protease inhibitor, as a protein with dual activity during the pollen rejection response. For example, in an incompatible cross, NaStEP would be inhibiting the protease that degrades HT-B. In its second function, NaStEP would be translocated into the PT mitochondria, where it interacts with NaSIPP, a mitochondrial phosphate carrier. This interaction would cause a mitochondrial destabilization that would lead to release of factors that promote programmed cell death.
Thus, under our hypothesis, both events would lead to the vacuole disruption as a terminal event of PCD. Once in the cytosol, S-RNase would not be active until NaTrxh, a thioredoxin h, reduces one of their disulfide bonds. On the contrary, in a compatible cross, NaStEP would be degraded, inactivated or sequestered, to avoid the rupture of the vacuole with the S-RNases and preventing the TP from dying.

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MINISIMPOSIUM 3: REGULATION OF GENE EXPRESSION

Evolution and function of fibrillarin in plants

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Fibrillarin is a nucleolar methyltransferase with essential functions for life. It is localized primarily in the nucleoli and Cajal bodies of eukaryotic cells. The main role stablish for this protein is the methylation of rRNA for proper ribosome assembly, as well as, methylation of histone H2A in the promoter region in rRNA genes. Furthermore, this protein is required for several viral particles for their progression in plants and animals, including humans. The apparent conservation of this protein has blindly left missing the fundamental information that an in-deep analysis could provide for the evolutionary dynamics of fibrillarin. Here, we applied a novel synteny network approach coupled to phylogenetic profiling to understand the genomic context of fibrillarin (hereafter 'Fib' is used as synonym) through principal lineages.
We identified 1067 non-redundant proteins across 966 completed-sequenced genomes across the tree of life. Synteny network analysis showed that Fib genomic context is conserved through plants evolution. Thanks to the rapid increase in genomes sequencing, today we have evidence of different genome evolutionary processes at small-sacale (tandem, proximal, segmental, transposed-mediated duplications, gene loss) and large-scale (Whole-genome duplication and triplication, WGD/WGT respectively; also know as polyploidy) that provide raw material for evolution and therefore for the biological diversity

A singular genetic trick in the course of evolution protects *Arabidopsis thaliana* from drought stress.

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Gene duplication is a frequent phenomenon that contributes to the processes of adaptation and diversification of species. In plants, multigenic families, have expanded and diversified from single ancestral genes throughout various duplication mechanisms. ATL gene family, a RING-H2 type ubiquitin-ligases (E3), is an example of gene family exclusive of plants. E3 enzymes are components of the ubiquitin-proteasome system, involved in the labeling of protein substrates for selective degradation. We study the promotor architecture of the duplC-ATL a sub-group of 6 duplicated *ATLs*. *AtATL78*, a member of duplC-ATLs, has been previously implicated in water deficit responses. A comparative structural analysis in Brassicaceae, indicates the occurrence of five TATA-box signatures within the promoter region of this group (up-TATA, TATA-0, TATA-1 TATA-2a and TATA-2b). Evolutionary history studies and analyses of gene expression of duplC-ATLs, suggest that up-TATA, TATA-0 and TATA-1, define an ancient zone that drives expression throughout reproductive tissues, and that TATA-2a and TATA-2b define a recent duplicated zone that drives expression throughout vegetative tissues. These analyses also revealed that TATA-2b was generated by a tandem duplication of a 30 nucleotides fragment, that only occurred within the promoter region of *ATL78* orthologs of lineage I of Brassicaceae. Remarkably, this duplicated TATA-box increases gene expression. A transcriptomic comparison of wild-type and *atatl78* mutant lines, strongly suggest that TATA-2b contributes to water deficit tolerance in *A. thaliana*.

Little-Big Guardians of Dedifferentiation: sRNA impact on Maize Somatic Embryogenesis

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Maize Somatic Embryogenesis (SE) requires calli induction with regenerative potential highly dependent on the explant and genotype. The molecular features underlying this potential are still poorly explored. To better understand the dedifferentiation response we characterized the morphology and small RNA (sRNA)-mediated gene expression regulation in maize embryos at different developmental stages during the Induction of calli tissues. Embryo explants showed contrasting phenotypes upon dedifferentiation and distinct embryogenic potential. The differential abundance of sRNA populations and that of their mRNA targets was explored by High-throughput Sequencing technology in the embryos before induction, during dedifferentiation and in the calli showing different regenerative potential. Early embryo developmental stage (15 days upon pollination) was characterized by the enrichment of development- and auxin- related sRNAs correlating with differential accumulation of Transcription Factors (TFs) possibly involved in the embryogenic potential during dedifferentiation. Transcriptomes provided the opportunity to analyze the complete battery of maize annotated TFs and functions by Gene Ontology Analysis. The dedifferentiation process from embryos with high regenerative potential was enriched in oxidation/reduction, transcriptional regulation and metabolic processes, as well as greatly represented by Plant hormone signal transduction and phenylpropanoid biosynthesis pathways. Particular TF modules such as Auxin Response Factors (ARFs) and others related to auxin production and signal transduction (YUC and HB) as well as embryogenesis-related SCRO1 and AP2-EREB were also characteristic of early embryos.
Overall, we conclude that particular sRNA and TF regulatory networks operating at 15 days upon pollination in the maize embryo are of outmost relevance to provide the required plasticity for dedifferentiation without losing the embryogenic potential, together with a proper management of auxin signaling and stress responses.

Functional analysis of geminivirus promoters for overexpression of recombinant proteins of pharmaceutical interest in *Chlamydomonas reinhardtii*

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The use of the green microalgae *Chlamydomonas reinhardtii* as a biological platform for the production of recombinant proteins is promising because of its high yields, low production costs, right processing of proteins and, specially, due to this organism is safe for human consumption. The employment of viral vectors for the increased production of heterologous proteins in microalgae, has a huge biotechnological potential, but is a research area scarcely developed. In the present work, we explored the functionality of several promoters from geminiviruses (higher-plant viruses) in *C. reinhardtii*, with the aim of determine their utility as part of a system based on viral genetic elements, which would integrate into the microalgae genome to subsequently split-out as an episome able to perform self-replication and multiplication. The *Rep* promoter from *Tomato Chino La Paz Virus*, the *Rep* and *CP* promoters from two variants of *Tomato Yellow Leaf Curl Virus*, with strong differences in their infective phenotype, were fused transcriptionally to distinct reporter genes, *GUS* and *GFP,* and integrated into expression vectors derived from the binary plasmids pB121, pCAMBIA and pChlamy. For the construction of the viral vectors we used synthetic oligonucleotides with unique sites for specific endonucleases. The generated vectors were experimentally analyzed in *Chlamydomonas* cells transfected by *A. tumefaciens* strains harboring the modified binary plasmids. The functionality and relative strength of the geminiviral promoters were evaluated by histochemical techniques, immunoassays, flow cytometry and confocal microscopy analyses. The evidence from histochemical and Dot Blot assays demonstrated the functionality of geminivirus promoters at microalgae; thus opening the possibility to use them for the construction of geminivirus-derived vectors for overexpression of recombinant proteins in microalgae.

Plant mitochondrial DNA replication: between recombination and origin dependent replication mechanisms mediated by unique enzymes

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Mitochondrial genomes vary in size and complexity, for instance their genomes DNA content vary in more than 3 orders of magnitude and it can exist as circular, linear, and mixtures of maxi and mini-circles. Plant mitochondria contains linear molecules, head-to-tail concatamers, branched, and rosette-like structures suggesting that single-stranded DNA directs primer formation in a mechanism similar to bacteriophage T4 recombination-dependent replication. However, plant mitochondria also harbor enzymes that resemble the bacteriophage T7, in which lagging and leader DNA synthesis are coordinated. The mechanisms that explain DNA replication in plant mitochondrial are unknown and furthermore they individual components have not been mechanistically characterized.
We have found that the unique DNA polymerases in plant mitochondria are able to bypass DNA lesions like abasic sites and thymine glycol, are able to participate in Base Excision Repair and perform microhomology-mediated end joining. Mitochondrial DNA polymerases coordinate with plant primase-helicase, suggesting that a canonical replisome is assembled in plant mitochondria. Plant mitochondrial replisome is an example of protein economy, in which unique inserts have evolved to confer novel functions to otherwise canonical polymerases.
We recently found a novel polymerase that localizes into nuclei, mitochondria, and chloroplast. This polymerase also harbors primase activity and maybe responsible to the wealth of genomic rearrangements present in plant mitochondria. This polymerase coordinates lesion bypass in combination with canonical plant mitochondrial polymerases during translesion DNA synthesis.
Our work is discovering how the enzymatic properties of DNA binding proteins in plant mitochondria correlate with the unique properties of plant mitochondrial genomes.

The complexity of folate polyglutamylation in plants: ripening and ethylene modulate polyglutamylated profiles in climacteric fruits plus systematic analysis of the glutamyl tail-editing enzymes

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Folate derivatives exist in nature in a variety of polyglutamyl forms (Glun), the glutamyl tail is added to the folate molecule by folylpolyglutamate synthetase (FPGS) and removed by gamma-glutamyl hydrolase (GGH) isoforms in a number of compartments within the cell. Folate polyglutamylation affects the use of the folate cofactors and their transport in organisms impacting also their bioavailability as vitamins in mammals; however, little is known about its regulation in plants. We profiled the polyglutamylation extent of the main folate in climacteric fruits, 5-CH_3-THF, and demonstrate that the profile is dynamic through ripening and it is affected by exogenous ethylene gassing being long Glun tails more susceptible to the treatment than fruits with short ones. In addition, we retrieved and compared deduced FPGS and GGH sequences from plants with known Glun profile and attempted to correlate their copy number, predicted localization, and primary sequence with the Glun profiles generated and gathered by this study. We present evidence of developmental, environmental and possible genetic factors impacting Glun extent in plants.

MINISIMPOSIUM 4: PLANT - MICROBE INTERACTIONS

The common bean –*Rhizobium etli* Nitrogen Fixing Symbiosis: deciphering novel regulatory pathways

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Common bean (Phaseolus vulgaris) is the most important legume for human consumption. As other legumes, common bean is capable of establishing symbiosis with N2-fixing soil bacteria known as rhizobia. Symbiotic N2-fixation (SNF) by differentiated bacteroids takes place in the rhizobia-induced root nodules. SNF reduces the cost of legume cultivation and is relevant for sustainable agriculture. Legume research throughout the world has contributed to the knowledge of different regulatory molecules, regulatory networks and signaling pathways essential for the establishment and function of SNF. However, there is still a lot to learn about this complex process that is finely and strictly controlled.

Our group has contributed to functional genomics of common bean, namely the transcriptome and the smallRNAome; and the genome-wide analysis of Alternative Splicing (AS) events in common bean and soybean. Derived from these data, our current research focuses on the analysis of selected novel regulatory molecules/processes proposed to be relevant for the control of common bean SNF at the transcriptional and post-transcriptional levels.

We are analyzing the role of selected members of the common bean MADS (AGL) –transcription factors (TF) family proposed to have a role in the transcriptional regulation of the SNF process. These AGL genes are highly expressed in inoculated roots and/or nodules. Their orthologs are also expressed in roots from Arabidopsis and other legume species.

We have demonstrated the essential roles of the microRNA (miRNA) –target genes nodes in common bean nodule development and function. These include the miR172c – AP2-1 and the miR319d – TCP10 nodes. We are exploring AS events that, based in our bioinformatics analysis, occur in common bean genes expressed in nodules and roots. We have experimentally validated such AS events and hypothesize that these process plays a role in regulating gene expression during the SNF symbiosis.

A *WRKY* transcription factor required for nodule development and symbiotic nitrogen fixation in *Medicago truncatula*

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Legumes are the third largest family of angiosperms and they are important for sustainable agriculture because they form nitrogen-fixing symbiosis with rhizobia. Many genes have been implicated in the nodulation process, including genes encoding transcription factors (TFs). Transcriptomic analysis of M*edicago truncatula*, a model legume, revealed that 417 TF genes are nodule-enhanced, including *WRKY* family although *WRKY* genes have not yet been implicated in SNF. Here, we report on the role of one of the nodule-enhanced *WRKY* genes, *MtWRKY1.* Two homozygous *wrky1 Tnt1* mutant lines of Medicago were isolated. Both were found to produce small white nodules unable to fix nitrogen with *Sinorhizobium meliloti* strain, Sm1021. Microscopy sections of *wrky1* nodules revealed aberrant nodule zonation in the mutant. Rhizobia failed to differentiate completely and to persist in the *wrky1* mutants.
Genetic crosses of *wrky1-1* and *wrky1-2* mutants yielded fix- progeny only, consistent with the conclusion that mutations in the *WRKY1* gene caused the fix- phenotype. On the other hand, progeny of *wrky1-1* or *wrky1-2* plants backcrossed with wild-type R108 plants exhibited Mendelian segregation, with a 3:1 wildtype to mutant phenotype ratio indicating that the underlying mutations were recessive. GUS activity in Medicago R108 roots and nodules transformed with $WRKY1_{promoter}$-GUS construct indicated that the *WRKY1* gene is expressed in root tips, infection threads, nodule infection zone and interzone. Transgenic hairy roots expressing *WRKY1* genomic sequence tagged with GFP indicated that WRKY1 protein is localized in the nucleus, consistent with its presumed role as transcriptional regulator. Furthermore, the fix- phenotype of the *wrky1* mutant was complemented to fix+ by transformation with *WRKY1* genomic sequence under the control of its own promoter. Transcriptome analysis of the *wrky1* mutants is underway.

Early Phosphorylated Protein1: A Novel Positive Regulator of the legume-rhizobia symbiosis

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Signals and signaling pathways underlying the symbiosis between legumes and rhizobia have been studied extensively over the past decades. In a previous phosphoproteomic study on the *Medicago truncatula* - *Sinorhizobium meliloti* symbiosis, we identified plant proteins that are differentially phosphorylated upon the perception of Nod factors. In this study, we provide experimental evidence that one of these proteins, Early Phosphorylated Protein 1 (EPP1), is required for the initiation of this symbiosis. Upon inoculation with rhizobia, *MtEPP1* expression was induced in curled root hairs. Down-regulation of *MtEPP1* in *M. truncatula* roots almost abolished calcium spiking, reduced the expression of essential symbiosis-related genes (*MtNIN, MtNF-YB1, MtERN1,* and *MtENOD40*), and strongly decreased nodule development. Despite that the down-regulation of EPP1 negatively affects essential components of the so-called Common Symbiosis Pathway, we did not observe significant effects in the symbiosis with arbuscular mycorrhizal fungi. Further investigation revealed that a phosphomitic mutant version of MtEPP1 is able to partially reestablish the symbiotic phenotype in the *M. truncatula dmi2* (a Nod Factor co-receptor) mutant plant. Altogether, these findings indicate that *MtEPP1* is essential to activate the common symbiosis pathway allowing *M. truncatula* interacts with rhizobia.

Exploring the roles of the *RALF-FER-RIPK* signaling during the symbiosis of common bean with rhizobia

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FERONIA (FER) is a plant membrane receptor like kinase, that participates in a variety of plant processes such as fertility, hormone signaling and polar growth, among others (1-3). In *Arabidopsis thaliana* roots, the cysteine rich peptide RALF interact with FER through its extracellular domain, which in turn, recruits the cytoplasmic kinase RIPK1, leading to the activation of both kinases (4). *RALF*, *FER* and *RIPK1* have been reported as essential for root hair growth (2, 4, 5), which are necessary to stablish a symbiosis process with rhizobia. Also, the three proteins are involved in pathogenic responses, that it is long known that share some pathways with symbiosis (6). RALF has been reported as necessary for an effective symbiosis in *Medicago truncatula* roots (7), but the exact mechanism, and whether the peptide is also required in determinate nodules, remains unknown.
Herein, the spatio-temporal activities of *FER* (*PvFER1*), two *RALF* (*PvRALF1 and PvRALF6*) and two *RIPK* (*PvRIPK2 and PvRIPK3)* gene promoters from *P. vulgaris* were evaluated during nodulation in transgenic roots. A clear promoter activity for each of the five genes studied was observed during the development of nodule primordia and in the nodule vascular tissue, suggesting the participation of these five genes in nodule organogenesis. Next, we explored the functional role of these 5 genes during nodule development through reverse genetics in hairy roots. Knock-down (KD) of Pv*RALF1*, Pv*RALF6* or *PvFER1* genes, resulted in a delay in the infection process generating fewer and smaller nodules when compared to control roots at early times of postinoculation. Over-expression (OX) of *PvRALF1, PvRALF6* or *PvFER1*, generated more nodules, however, while *PvRALF1* or *PvRALF6* OX roots had bigger nodules than the control, *PvFER1* OX roots developed smaller nodules. Nitrogen fixation was only slightly affected when any of the three genes were KD or OX. The reverse genetic analysis of *PvRIPK2* and *PvRIPK3* is still in process.

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Microbial volatile organic compounds in desert plants: identification, function and biotechnological application

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A growing number of bacteria and fungi have been found to promote plant growth through mutualistic interactions involving elements such as volatile organic compounds (VOCs). However, evidence of these molecules and their effects in plants from arid ecosystems is limited.

Here, we reported microbial VOCs produced by 62 core and representative members of the microbiome of agaves and cacti in their interaction with *Arabidopsis thaliana*.

Our study revealed that approximately 90% of the bacterial and 70% of the fungal strains promoted plant growth in *A*. *thaliana.* Bacterial VOCs were mainly composed of esters, alcohols, and S-containing compounds with 25% of them not previously characterized. Remarkably, ethyl isovalerate, isoamyl acetate, 3-methyl-1-butanol, benzyl alcohol, 2-phenylethyl alcohol, and 3-(methylthio)-1-propanol, and some of their mixtures, displayed beneficial effects in *A*. *thaliana* and also improved growth and development of *Agave tequilana* and *Agave salmiana* in just 60 days. Fungal VOCs were composed of sesquiterpenes, alcohols, aliphatic compounds, monoterpenes, ketones, esters, aldehydes, ethers, diterpenes, N-containing compounds and organic acids with 45% of them not previously characterized. Camphene and benzyl benzoate improved growth in *A. thaliana*, *A. tequilana* and *A. salmiana*.

To test the potential efficacy of mVOCs on agricultural productivity, *A. salmiana* and *A. tequilana* plants were pre-exposed to volatiles and planted in the greenhouse to monitor growth and biomass production. Results showed that treated agave plants increased 200% more root, stem and leaves biomass than untreated controls after
8 months (without exposition). Altogether the data suggest that volatiles produced by bacteria and fungi isolated from agaves and cacti are promising molecules for the sustainable production of crops in arid and semi-arid regions.

Study of a plant – pathogen – endophyte interaction: the case of maize – *Fusarium verticillioides – Bacillus cereus B*25

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Fusarium verticillioides (*Fv*) is a fungal pathogen of maize – one of the most important grains worldwide – that affects not only corn but also animal and human health. The use of the rhizospheric bacterium *Bacillus cereus B*25 has been reported as a biocontrol agent to prevent *Fv* maize colonization in the field due to the ability of *B*25 to produce siderophores, proteases and two chitinases, which disturb the growth of the fungus and controls the pathogen.

The underlying molecular mechanisms responsible for the antagonism during this bacteria-fungus-plant interaction remain to be determined. In order to do so, we first developed an efficient transformation protocol to enable genetic manipulation (to delete the bacterium genes related to its activity against *Fv*). Next, we aimed to track both the bacteria and *Fv* inside the maize root system and finally, to study the expression of *B*25 genes related to the antagonism of *Fv.*

We have successfully developed a transformation protocol that allowed us to transform *B*25 with a plasmid carrying a green fluorescent protein to trace the bacteria inside the root system of maize. As for the study of gene expression, by qPCR analysis, we have seen an induction of several genes related to the *B*25-*Fv* interaction, such as those coding for *B*25 siderophores and chitinases.

Bacterial chitinases are possibly implicated in restoring the plant defense mechanism mediated by plant chitinases. This plant-mediated fungal invasion surveillance mechanism is lost due to the activity of *Fv* effector proteins that cut plant chitinases. Bacterial chitinases are resilient to *Fv* effector proteins and remain active. We are interested in generating *B*25 mutants of the chitinase-coding genes involved in the antagonistic mechanism to fully understand the role they play in this tripartite interaction and we are currently working towards generating *B*25 chitinase mutants.

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Unveiling the microbiome of *Haplaxius crudus:* the coconut lethal yellowing phytoplasma vector

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Haplaxius crudus is the only insect that has been confirmed as a phytoplasma vector (‘*Candidatus* Phytoplasma palmae’; 16SrIV group) that cause the disease known as coconut Lethal Yellowing (LY). Its reproductive success and evolution could depend on beneficial microorganisms that enriched poor diets with nutrients, helping in digestion of recalcitrant food, providing protection against predators, parasite, and pathogens, manipulate the mating and reproduction systems, but, more importantly, may affect their efficiency as LY vector. In the present study, we unveiled the richness and abundances of the microbial community associated with *H. crudus* using high-throughput sequencing targeting 16S rRNA gene. In eight samples sequenced, we obtained a total of 1 305 206 raw sequences, after removal of adaptors and chimeras we retained 589 624 sequences of good quality. The detected sequences belong to 11 phyla, the most abundant group was Bacteroidetes (60%) followed by Proteobacteria (22%) and Tenericutes (2%). Five out of eight insects sequenced were positive for LY with 6 534, 6 124, 1 136, 43 and 25 reads per insect. Phylogenetic analysis of LY phytoplasma revealed that three insects carried phytoplasma of the subgroup 16SrIV-A and two insects carried phytoplasma of the subgroup16SrIV-D. Phytoplasma of the 16SrIV-A showed more reads (6 534, 6 124 and 1 136 reads per insect) than subgroup 16SrIV-D (43 and 25 reads per insect). The results obtained in the present work contribute with three important points to deal with the problematic of LY: 1) provided important information with the role that plays the microbiome during the palm-insect-phytoplasma interaction, 2) bring possibilities for the identification of microorganisms with biotechnological potential for the biological control of phytoplasma, and 3) opened a new field of research in phytopathology of the most important diseases of coconut.

MINISIMPOSIUM 5: STRESS RESPONSES AND CLIMATE CHANGE

Carbon metabolism in bean pods during seed development

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The pods of legume fruits are not merely protecting structures, they also play a crucial role in regulating carbon partitioning to the developing seeds. Pod walls are photosynthetically active and contribute to fulfill the needs of developing seeds, and simultaneously perform the action of active sinks. To understand the role of the *P. vulgaris* pod through the seed growth, we assessed the changes in glucose, fructose, sucrose and starch during fruit development. The results show that carbohydrates are transiently stored in the pod, and efficiently used during seed development. The incorporation of $^{14}CO_2$ shows that the photosynthetic activity of fruit and source leaf contributes to the carbohydrates present in the pod. However, the carbon fixed by the pod is more efficiently incorporated within the starch, while larger proportion of the $^{14}CO_2$ fixed by the source leaf is translocated towards the developing seeds. Our results also show differences in the photosynthetic activity between genotypes of bean pods as well as in the amount of starch present in this organ. Chlorophyll levels and ADPGase and Rubisco activities were not related with the amount of starch in the pods or with its ability to fix $^{14}CO_2$. However, both variables are clearly dependent on the amount of pod open stomata. To define the function of the carbon assimilated by the pods, bean fruits were detached from the plant and exposed to a severe nutritional restriction. Under these conditions, the starch in the pod was almost completely removed after 5 days. Pod resources were insufficient to support the needs of all seeds. However, by the accumulation of callose in the sieve plates at the funiculus level, the resources were channeled to a few seeds that successfully completed its development. Finally, we hypothesize that the accumulation-recycling of starch in non-conventional organs, such as the pods, might be involved in supporting seed development when photosynthates supply is reduced as a consequence from unexpected adverse environmental conditions.

This research was supported by DGAPA-UNAM (PAPIIT IN203017) and Facultad de Química (PAIP 5000-9127).

Analysis of the hydrotropic root response and elongation of mesocotyl in DTMA (Drought Tolerant Maize for Africa) hybrids under low water potential gradient conditions.

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Radical system and the mesocotyl are two belowground structures that play a critical role for the emergence and seedling growth of maize. The first trait is the main responsible for the absorption of water and nutrients for plant growth and development, while the second is a basic structure of the stem, responsible for pushing the coleoptile to the soil surface. Both structures are fundamental for the establishment and seedling vigor of the plant, they interact in darkness and their phenotypic-genotypic variation could be associated with the adaptive response to water limitation conditions generated by current climate changes. These agronomic features of maize had to withstand drought stress affecting agricultural productivity, diminishing crop yield and food security for a constantly growing population. In this study, we examined the relationship between the root hydrotropic response and mesocotyl elongation under low water potential gradient conditions. The phenotypic analysis of the traits was developed in 72 DTMA hybrids with their hydrotropic responses known of a population of 285 under an experimental system designed for both traits by a period of 7 d. According to the phenotypic observations, both traits were statistically correlated, and they showed positive correlations with others plant growth traits (primary root, leaf and shoot length). With the major access to next-generation sequencing technologies, we used *Genome-Wide Association Studies* (GWAS) as a powerful tool for the locating and associating of genes to complex traits. The GWAS was developed with 335,391 SNPs to two traits, large scale associations explaining more than 35,15% of the genetic variance were detected, 172 SNPs and 90 SNPs were identified to be highly associated with hydrotropic response of primary root and the mesocotyl elongation with p-values >3,25. The SNPs were distributed along of the 10 chromosomes in the two traits. However, for mesocotyl elongation they were concentrated in the chromosome 6. We identified 89 genes associated to hydrotropic response and 48 genes associated to mesocotyl elongation. We found genes that have been described in responses to the abiotic and biotic stress as the Harpin-induced protein (LEA) and the GRAS76 chitin-inducible gibberellin-responsive protein. We will continue to analyze of potential interactions between the genes and their co-expressions by RNAseq. Our study will generate new knowledge of the importance of the study of the root system and mesocotyl elongation in relation to maize tolerance to drought and in its analysis as an assisted selection marker in breeding programs.
This research was supported by DGAPA-UNAM: IN214119.

Molecular and ecophysiological characterization of abiotic stress responses in *Marchantia polymorpha*.

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Plants rely on a suite of molecular and cellular mechanisms for the rapid perception and response to biotic and abiotic stresses. Salt stress -mainly sodium chloride (NaCl)- and thermal stress are two of the most common abiotic stresses affecting plant fitness and production in the world. In order to understand the function and evolution of the molecular response modules involved in salt and thermal stress repsonses, we employed *Marchantia polymorpha*, one of the earliest diverging land plant that colonized the landscape ~470 millions of years ago, as our model. We established assays to induce salt and thermal stresses during the gametophytic phase of *M. polymorpha* and then characterized the immediate -2 hours (2 h)- and early (24 h) salt stress response (100 mM of NaCl) at the transcriptional (RNA-seq) level. We found that *M. polymorpha* copes with salinity stress through a common response module shared across both time points that consist in the upregulation of genes involved in carbohydrates, amino acids and lipid biosynthesis in order to maintain cell integrity and basal functions. In the case of thermal stress, we identified the repertoire of heat-shock transcription factors (Hsf) that act as master regulators of the thermal stress response in land plants and found that overexpression of one of the two members of the MpHsfA clade, results in increased biomass production and increased thermotolerance.

Analysis of genetic variation associated to hydrotropism and deep planting resistance in maize (*Zea mays* L.) revealed genes controlling response to drought, growth, development and adaptation to global heating.

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Maize adaptation to drought and high temperatures depends not only on genetic and phenetic traits but also with their interaction with the physical, chemical, and biological environments. To better understand these complex interactions, we studied the hydrotropic response of roots and deep planting resistance (higher elongation of the mesocotyl) in inbred lines, hybrids, and native maize landraces of Mexico. The root hydrotropic response (HR) of primary roots of maize hybrids, if robust (with curvatures larger than 40 degrees), the mature plant tolerates severe drought, partial irrigation and higher temperatures. The root system architecture of seedlings with robust HR change by producing less crown roots with increase aerenchyma and small cortex cells, which enhance both uptake of water and foraging for water /nutrients by reducing the cost of respiration. Deep planting is practiced in arid regions of Mexico and the US southwest to take advantage of residual soil humidity. Deep planting depends upon the ability of the mesocotyl to increase elongation (~20-30 cm) and push the cotyledon to the light. We performed Genome Wide Association Studies (GWAS) of the hydrotropic response and resistance to deep planting under drought conditions in 283 hybrids of maize. We have identified by GWAS several genes and phenes implicated in the regulation of root hydrotropism and elongation of mesocotyl submitted to drought. We have also made RNAseq studies of the earlier root hydrotropic response in maize inbred lines and identified several expressed genes that orchestrated the control of cell division, cell growth, response to drought and degradation of proteins by the 20S proteasome. The identification of these molecular markers will be fundamental to understand the evolution of traits relevant to drought and higher temperatures adaptation in times of climate change and global heating.

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Chromate treatment of *MEDIATOR* mutants triggers the splitting of the root meristem in *Arabidopsis*.

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The twinning of the root apical meristem following damage has been observed in cut root tips of maize, tomato and in *Arabidopsis*, where root splitting occurs without external damage in mutants of cell-cycle regulators. Here, we present a twinning root phenotype that arises when *Arabidopsis* plants are genetically compromised at the *MED18* locus, which sensitizes the roots for DNA damaging factors combined with chromate treatments, a DNA damage inducing metal. In the *med18* mutants, *ERF115*, a factor that promotes cell renewal after stem cell loss, is strongly up regulated in the root meristem. The quiescent center gene marker *WOX5* and auxin-related gene expression traced the changes in stem cell identity and were induced in a *MED18* and chromate-dependent manner going from one to two meristems, and this process required auxin redistribution and signaling mediated by *IAA14/SOLITARY ROOT (SLR1).* Thus, duplication of the root meristem allowed dichotomous root branching in *Arabidopsis*, a developmental program thought to be specific of the ancestral lineages of plants. Moreover, our work unravels a mechanism by which upon injury, cells change their fates to drive full organ regeneration leading to an extreme and unusual developmental program.

Metabolic response to larval herbivory in *Physalis sp*.

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Insect herbivory response in plants rely on many metabolites with known defense function. These compounds can be constitutively active or induced following herbivore attack, through complex reorganization of primary and secondary metabolism. Metabolite defense depend mostly on plant host, insect herbivore, type and intensity of damage, among other factors. This research aims to analyze metabolic fingerprinting of three species from the genus *Physalis* (*P. angulata*, *P. grisea* and *P. philadelphica*) after larval herbivory (*Trichoplusia ni*) in order to compare herbivore response through phylogenetically related plant species and recognize host specific herbivory biomarkers. For this purpose, untargeted metabolic approach was carried out on leaf material of each *Physalis* species using UPLC-MS and analyzed by multivariate statistics (PERMANOVA, PCA, PLS-DA and OPLS-DA). A total of 103 features were selected in positive mode for the three plant species. PERMANOVA and PCA analyses of metabolic fingerprints indicated significant differences among plants species and herbivory condition. Biomarkers for plant species (7) and herbivory (6) were identified by PLS. In general, abundance in metabolic signature was higher for *P. philadelphica*, which shared more common features and abundance with *P. grisea*. Larval herbivory altered plant metabolome in all three species, highlighting *P. angulata* with changes in 44% of its profile. OPLS- DA identified 29 herbivory biomarkers and the hierarchical data structure of these biomarkers indicated similar profiles in *P. philadelphica* and *P. grisea*. The untargeted metabolomics approach used in this study contributes to explore and understand phytochemical composition of *Physalis sp.* under herbivory.

MINISIMPOSIUM 6: EPIGENETICS

The *Arabidopsis* methylome is robust to dark-induced senescence

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In plants, senescence is part of the normal developmental program to dismantle the leaves and remobilize nutrients to the seed, ensuring reproductive success (1). At the molecular level, developmental plant senescence is accompanied by a global transcriptional reprogramming and changes in the chromatin configuration that include decondensation of heterochromatin at chromocenters and relocation of the silencing histone marks H3K9me2 and K3K27me2 (2). We found that decondensation of the chromocenters is a nuclear phenotype common to senescence, since we observed the same expansion of heterochromatin in interphase nuclei of senescent leaves induced by darkness as described in (3). Furthermore, we unveiled a global downregulation of a network of genes preserving the integrity of the chromatin. This downregulation was accompanied by deregulation of TEs, with young insertions being preferentially affected. Those changes however, were not accompanied by global disruption of the methylation landscape. In all three contexts, methylation levels remained stable along genes and TEs. Analyses at a higher resolution revealed differentially methylated regions (DMRs) to be most prevalent in the CHH context. Moreover, the presence of a senescent CHH DMR in the promoter of protein coding genes correlated with changes in expression, indicating that senescence-induced CHH methylation changes have a regulatory role. Together, this data reveal that the terminal stage of plant life is accompanied by global changes in chromatin structure, but localized changes in DNA methylation, adding another example for the dynamics of DNA methylation during plant development.

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The epigenetic behind the albinism in Agave

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Albinism in plants is a rare phenomenon that occurs in nature and is characterized by the total or partial loss of photosynthetic pigments. Although progress has been made in understanding the nature of this phenomenon, the epigenetic and biological basis are still unexplored. During the micropropagation of *Agave angustifolia* Haw., we found three different phenotypes, green (G), variegated (V) and albino (A). To understand the physiological and epigenetic differences among the Agave somaclones, we analyzed several morphophysiological parameters and changes in the DNA methylation patterns in the three phenotypes during their *in vitro* development. Epigenetic analysis revealed that global DNA methylation increased in the G phenotype during the first two subcultures. However, after that time, DNA methylation levels declined. This hypomethylation correlated with the appearance of V shoots in the G plantlets. A similar correlation occurred in the V phenotype, where an increase of 2 % in the global DNA methylation levels was correlated with the generation of A shoots in the V plantlets. We also analyzed different histone marks (H3K4me2, H3K36me2, H3K9ac, H3K9me2 and H3K27me3) and almost a 2–4.5-fold increase in H3K9ac was observed in albino plants in comparison with variegated or green plants, suggesting a change in chromatin compaction related to *A. angustifolia* albinism. This suggests that an ‘‘epigenetic stress memory’’ during *in vitro* conditions causes a chromatin shift that favors the generation of variegated and albino shoots.

RNA methylation or the epi-transcriptomic control of gene expression in the moss *Physcomitrella patens*

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There are over 150 chemical modifications that can occur in RNA. Among these, the most frequent internal modification in mRNA corresponds to methylation of position N6 in adenosines (m6A) at selected consensus sites. In recent years, m6A in animal systems has been described as a dynamic label that affects the fate of modified mRNAs, regulating mRNA splicing, stability, transport, translation, among other processes. In *Arabidopsis thaliana*, a handful of studies have uncovered the methyl-transferase complex components, demethylases and importantly a few m6A-binding factors that are responsible for its recognition and for directing the outcome of RNA methylation. The moss *Physcomitrella patens* is a model for the early evolution of land plants and contains numerous advantages to perform genetic and molecular analyses. In this plant we have identified and characterized the main factors of the methyl-transferase complex: METTL3, WTAP and METTL14A/B. Single or quadruple null mutants obtained through genome-editing of the corresponding genes are devoid of m6A modification of mRNA. Remarkably, key developmental steps are affected in these mutants, namely the fundamental transition from the bi-dimensional growth mode present in the protonemata to generate 3D structures known as gametophores is delayed in the mutants and thus, subsequent developmental stages are also affected. More dramatically, while sporophyte formation is achieved the resulting spores are not viable and fail to germinate in all mutants tested. This phenotype is reminiscent of defects seen when the METTL3 or WTAP genes are disrupted in Arabidopsis, rice, yeast and animals. Currently, we are exploring the identity of m6A-modified mRNAs present during the 2D to 3D transition to identify the relevance of methylation in this process. Also, we are studying the m6A binding factors present in *P. patens* to characterize their function during plant development.
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Female gametogenesis, a pathway mediated by *MIR822* regulates germline linage in *Arabidopsis thaliana*

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Flowering plants have two generations, a sporophyte or diploid and a gametophyte or haploid. The establishment of the haploid phase occurs when gametophytic precursors are specified and, in female tissue starts when a somatic subepidermal cell acquires Megaspore Mother Cell (MMC) identity. In *Arabidopsis thaliana* the MMC enters to meiosis and forms four haploid products, three of which degenerate and only one survives, this haploid cell acquires the identity of Functional Megaspore (FM) in a process so called monosporic. To date, it is unknown what are the molecular mechanisms that regulate the monosporic development in flowering plants. Here, we show that the *MIR822* gene that encode a miR822a is specifically expressed in the developing ovules, the *loss-of-function* mutant of *MIR822* causes that two haploid products survive instead of one, both surviving cells acquire FM identity. Whereas the extra FM-like cell is able to divide, is not able to form a fully differentiated female gametophyte, instead a multinucleated embryo sac is formed. The validation of target genes of miR822a showed that three genes of the same family are negatively regulated by miR822 and ARGONAUTE9 protein. Independent overexpression of each target gene, phenocopy the *mir822*. Taken together, these findings suggest a pathway mediated by *MIR822* that regulate the elimination of the haploid products in female gametogenesis and as a consequence, modulate the monosporic development in *Arabidopsis thaliana*. The evidence suggests a new role of miRNAs in the control of female sporogenesis in angiosperms.

Biotechnological implications of the analysis of plant meiotic mutants.

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Meiosis is a crucial step in sexuality allowing new genomic combinations fundamental for breeding programs. Our work intends to understand the molecular basis of the mechanisms that regulate the initiation of meiotic recombination. We are using maize, an historical plant model in cytogenetic study of meiosis. We have identified maize meiotic mutants altered in the initiation of meiotic recombination. The spo11-1 mutants do no form the normal ten bivalents due to defect in DNA Double Strand Break (DSB) formation. The analyses of chromosomal axial elements such as AFD1, ASY1 and DSY2 in the spo11-1 mutant reveal conformational remodeling of the meiotic axis during meiosis. Though meiotic mutants are generally sterile we will describe a strategy that allow a fertility rescue. Using these mutants, we can take advantage of the inhibition of the natural random meiotic recombination process in order to design the sites of meiotic recombination. This new strategy of directing meiotic recombination could have a revolutionary impact on plant breeding.

ptxD/Phi as a dominant and stable selectable marker system for cyanobacteria and microalgae

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Microalgae and cyanobacteria are photosynthetic organisms with a high biomass generation capacity and with very simple growth requirements, positioning them as the perfect bioreactors for the generation of value-added products and also for the study of biological fundamental topics. Therefore, genetic transformation is usually required to accomplish different purposes. Currently, antibiotic and herbicides resistance-based selectable markers are used for research and industrial applications. However, these agents are expensive and their effectiveness to restrict the growth of non-transformed cells varies among species. Moreover, although microalgae and cyanobacteria are GRAS (Generally Recognized As Safe) organisms, there are major concerns about the use of antibiotics and herbicides to produce cultures intended for food and feed. Therefore, the development of novel selectable marker systems effective for a broad range of cyanobacteria and microalgae that facilitate and increase the efficiency of genetic transformation and increase the stability of the gene of interest is of growing importance. Here, we report an effective and low-cost selectable system based on providing cyanobacteria and microalgae cells the capacity to convert a non-metabolizable compound (phosphite, Phi) into an essential nutrient for cell growth (phosphate) through the expression of a phosphite oxidoreductase. Our results demonstrate that this system is highly effective as a dominant selectable marker for the genetic transformation of *Synechococus elongatus*, *Chlamydomonas reinhardtii* and *Chlorella sorokiniana*. Transgenic lines are able to grow on media supplemented with Phi as sole source of phosphorus and selective agent. Phi is a much lower price and stable chemical than antibiotics commonly used as selective agents for the genetic transformation of microalgae and cyanobacteria. Phi is also considered by the FDA as a safe chemical for human and animal health. Therefore, the ptxD/Phi system could be implemented as a dominant and stable selectable marker in multiple microalgae and cyanobacteria to accomplish research and industrial purposes.

POSTER SESSIONS

DEVELOPMENT

001. Improvement of polyclonal antibody production as a tool for protein analysis in plants

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Antibodies are a powerful tool in life science research. All forms of antibodies, polyclonal (pAb), hybridoma-based monoclonal (mAb) and recombinant monoclonal (rAb), have both pros and cons as research tools and have features that differentiate them from one another. pAb have a number of advantages over the mAb, they typically recognize multiple epitopes on a target protein, so, they are more effective for detection of targets present at low quantities. For example, in chromatin immunoprecipitation (ChIP) and immunohistochemistry (IHC), pAbs are useful, since epitopes may be masked or modified by some treatments in the sample, as crosslinking and blocking. Additional benefits are the inexpensive and faster production, compared to mAb, and that they can be generated in a wide range of host animals. Here, we present a useful and reliable method for pAb production specific against plant proteins or peptides, which minimize the disadvantages inherent to pAbs. Some of the key steps are the purification of the recombinant proteins used for immunization, the antibody purification using immunoaffinity and additional steps of negative adsorption to eliminate contaminants. As examples, we show some pAb raised against maize proteins and their use in assays including Western blot and immunostaining to analyze protein null mutants and target protein localization *in situ*. As well as immunoprecipitation followed by mass spectrometry analysis to identify interacting protein complex. Overall our methods will help to address troubleshooting in antibody raising to explore the function of plant proteins with no commercial antibodies available. pAbs production service using these methods is currently being offered by the National Laboratory of Agricultural, Medical and Environmental Biotechnology (LANBAMA) at IPICYT.

002. Search for target genes of the transcription factor *BOLITA* in *Arabidopsis thaliana*

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Plant organogenesis keeps active after the embryo has germinated. This intriguing capacity confers these sessile organisms the ability to respond against an always changing environment. There are some factors associated to organogenesis in plants, like Auxin response maxima, where the efflux of auxin defines the specific sites where the organs will develop following the rules of phyllotaxy, but recently a transcription factor named *BOLITA (BOL)* has been associated to the start of organogenesis. The AP2/ERF transcription factor BOL, belongs to the family ERF in the Class VIII-b, this group includes other transcription factors like *DORNRÖSCHEN* and *LEAFY PETIOLE*, that are key regulators of plant development. *BOL* expression marks all the founder cells and it maintains during the development of primordia that will develop into new organs, but the molecular mechanism associated with this acquisition of cellular identity are still unknown. One approach to elucidate potential functions, is to identify target genes. In order to define potential target genes of *BOL* we analyzed transcriptome data obtained after *BOL* induction. We selected some genes and identified potential sites of interaction mediated by a GCC-box in the promoter regions. To validate the putative genes, we performed chromatin immunoprecipitation (ChIP) experiments using an Anti-GFP antibody, on vegetative and floral stages in a loss of function *BOL* mutant (*drnl-2*) harboring the *pBOL:BOL:GFP* construct that recovers the WT phenotype. Finally, we assessed the enrichment of potential interaction sites in the regulatory regions of putative target genes by qPCR.

003. Comparative 2-DE proteomic analysis of *Coffea canephora* high competitive and non-competitive pretreatment for somatic embryogenesis

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Somatic embryogenesis (SE) is a cell differentiation process by which some of the somatic cells switch into embryogenic cells. Those cells, later produce somatic embryos with the ability to generate plantlets. Given that competent-embryonic cell formation displays gene expression changes and the production of small molecules, proteins inventory might be altered in cells undergoing differentiation to somatic embryos. We utilize the SE process of *C. canephora* that yield a high number of embryos as a model to find early regulated proteins involved in the competent-embryonic cell formation. We determine proteins with up- and down abundance during pretreatment of the SE. We compared protein samples from pretreatment, which include growth regulators in the medium, with non-competitive pretreatment that lack growth regulators in the media. The identification of these proteins could help to insight into the understanding of the embryogenic competence of the cells in *C. canephora*.

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004. Distinguishing sexuality from apomixis in *Boechera stricta*

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Apomixis is an asexual method of reproduction by which some flowering plants generate clonal seeds. In contrast with sexual reproduction, apomixis is characterized by unreduced female gamete formation, fertilization-independent embryogenesis, and functional endosperm formation. Apomixis is currently perceived as a biotechnological strategy that could result in the fixation of heterosis through the production of selfperpetuated clonal hybrids; however, natural apomixis does not occur in any major crop species. *Boechera stricta* is a member of the *Brassicaceae* in which natural accessions can reproduce sexually or by apomixis, although the unequivocal description of the developmental mechanisms occurring in its ovules has yet to be conducted. We developed cellular, genetic and molecular studies to compare female gametogenesis and seed formation in selected diploid genotypes of *Boechera stricta* that reproduce either sexually or by apomixis. A detailed analysis of female gametophyte formation indicates that *Boechera stricta* exhibits aposporous development and accelerated megasporogenesis, as compared to sexual development in the same species. We found a link between epigenetic regulation and apomixis by comparing the *in situ* expression pattern of selected *ARGONAUTE* (*AGO*) genes involved in *de novo* DNA methylation during either sexual or apomictic ovule development. We also developed a simple genetic transformation procedure that allows the recovery of transgenic plants harboring reporter genes under the control of specific gametophytic promoters of *Arabidopsis thaliana*, a relative of *Boechera* sp. Our results establish a model system in which sexuality and apomixis are highly penetrant in independent genotypes that can be used for molecular and genetic studies to establish the basis of natural asexual seed formation.

005. The kinase activity of maize D type cyclin/cyclin dependent kinase complexes is regulated by the subcelular localization during germination

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Seed germination begins with the uptake of water by the dry seed and ends with the radicle protrusion. Radicle protrusion is an event that not necessarily involves cell division but requires cell cycle establishment for the growth and development of the new plant. In plants, as in mammals, the association between a cyclin (Cyc) and a cyclin dependent kinase (CDK), determines cell cycle progression. Information about cell cycle regulation in plants has increased, however, most of the studies are centered on gene expression or in the accumulation of Cycs and CDKs, but little is known about their subcellular location. In this work, we implemented a protocol for nuclei isolation that achieved separation of nuclei from cytoplasm. First, we evaluated the location of D type cyclins and CDKs in both nuclear and cytoplasmic fractions by western blot using specific antibodies. Cycs D3;1, D4;2, D5;3, CDKA and CDKB1;1 are present in both fractions during germination. Subsequently, we investigated the complexes formed by these proteins through immunoprecipitation assays. Our results showed that the three CycsD are associated with CDKA in both nuclear and cytoplasmic fractions while their association with CDKB1;1 is predominantly observed in the cytoplasmic fraction. Immunoprecipitated CycsD/CDKs complexes were tested for kinase activity on two substrates, histone H1 and the retinoblastoma-related protein. Complexes from both fractions can phosphorylate the two proteins in a differential manner depending on germination time. Finally, using a methodology to extract chromatin-associated proteins, we extracted CycsD/CDKs complexes with higher kinase activity than in the complexes in cytoplasm or in nuclear fractions, apparently due to the presence of a phospho-activated CDK, suggesting the existence of different complexes in each cell compartment.

006. Filter aided sample preparation (FASP) coupled to mass spectrometry leads deep proteome: *Musa acuminata* ssp. *malaccensis* (Selangor) male flower and female flower

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Edible banana fruits consumed across the world are vegetatively parthenocarpic; seedless fruits developed without the stimulus of pollination. By contrast, the wild ancestors of edible bananas are fully seeded, their fruit developed if pollinated. Common primordia were undergoing cell differentiation process to generate the female (pistillate) and male (staminate) flowers. The contrasting features of those flowers, stamens lacking filaments and anthers in the female flower but developed ovaries, and functional filaments and anthers but poorly development ovaries in male flower, might be supported by an inventory of proteins. We utilize FASP-based protein samples to determine an inventory of proteins from petals, flowers without petals, and complete flower of both pistillate and staminate flowers of *Musa acuminata* ssp. *malaccensis* (AA genome, ITC 1060) that could help to advance in the understanding the cell differentiation process in banana flowers.

007. Analyzing the function of SHOOT MERISTEMLESS (STM) related to cytokinin during gynoecium development in *Arabidopsis thaliana*

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The flower is a complex and beautiful structure, exclusive for the angiosperm group, and is formed by different floral organs including the reproductive organs. The gynoecium is the female reproductive organ, which once fully developed and fertilized forms a mature ovary or fruit. In Arabidopsis, the phytohormone cytokinin plays an important role in the early development of the gynoecium, mostly because it regulates cellular division in the meristematic region of the carpels; however, it has also functions during later development. Cytokinins are produced by an enzymatic pathway that uses ATP or ADP to convert them in a free and active base. The enzymes ISOPENTENYL TRANSFERASE (IPT) and LONELY GUY (LOG) play a very important role for this biosynthetic process. Each one of the IPT and LOG enzymes has nine genes coding for enzymes of their kind. When cytokinin is produced, it has to be sensed by the cytokinin receptors (AHK) to trigger its genetic response. It has been reported that the transcription factor SHOOTMERISTEMLESS (STM) plays a role related with the cytokinin production and perception by activating the expression of some of *IPT* and *AHK* genes that produce and perceive cytokinin, respectively. However, this function has been attributed to STM based on studies in seedlings. Since cytokinin and STM are very important for gynoecium development; and all the genes for biosynthesis (*IPT*; *LOG*) and perception (*AHK*) genes seem to be expressed in this structure, we want to know if STM regulates biosynthesis and perception of cytokinin in the gynoecium. In this study, we used genetic and molecular tools to determine the relation between STM and cytokinin production and perception, and to know if this regulation is direct or indirect. The latest results will be demonstrated.

008. The role of NaStEP in pollen rejection: a Kunitz type protease inhibitor with a dual activity as proteinase inhibitor and voltage channel blocker

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Self-Incompatibility (SI) is a genetic mechanism that favors diversity in many angiosperms by rejecting self-pollen. In Solanaceae, SI is based on the interaction between the female determinant, S-RNase, and the male determinant, SLFs. Other genes are known to participate in SI as well. One of these is NaStEP, a Kunitz-type proteinase inhibitor expressed by stigmatic cells from self-incompatible *Nicotiana* species. Although the precise role of NaStEP in SI has not yet been clarified, loss of function assays in *Nicotiana* transgenic plants indicate that NaStEP is essential for SI. Besides, our data show that in an incompatible cross, NaStEP is a positive regulator of HT-B stability, a protein that is presumably responsible for the S-RNase release from the vacuole inside pollen tubes.
Because some members of the Kunitz type proteinase inhibitors family possess activity as voltage channel blockers, we tested whether NaStEP exhibits this activity. To evaluate this, we incubated *Xenopus* oocytes that overexpress the Kv 1.3 potassium channel with native NaStEP purified from *N. alata* styles. The electrophysiological results show that NaStEP behaves as a channel blocker, which irreversibly binds to the Kv 1.3 potassium channel in a dose-dependent manner.
Regarding NaStEP as a proteinase inhibitor, we incubated NaStEP with the Ser-protease subtilisin and observed a proteolytic activity inhibition, but when we analyzed the integrity of NaStEP in presence of subtilisin, two processed forms of NaStEP were evident, one of that loses its C-terminal domain and a second form that is presumably inactive because the Kunitz domain is lost. Thus, we propose a suicide-like inhibition mechanism between subtilisin and NaStEP and discuss how it fits in the biochemical mechanism of S-specific pollen rejection in *Nicotiana*.

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009. Maize scutellum importance and its biological and agronomic impact

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Introduction. Cereal grain is a fruit containing a seed, this contains an embryo. Maize embryo is constituted by an embryo axis and an scutellum. Among cereals, maize scutellum is one of the biggest, and accumulate reserves as lipids, phytate, and some ions as zinc and calcium. During early germination embryo metabolism depends on stored nutriments of embryo axis and scutellum. The proportionally larger scutellum provides adaptive advantages: during germination the scutellum contributes with nutriments necessary for the reactivation of the embryonic axis metabolism which sustains embryo root protrusion. During postgermination it secretes enzymes and phytohormones, then it could absorb hydrolysis products and nutrients from the endosperm. The present study used Chalqueño maize, a creole maize with high productivity. *Material and Methods*. The aim of this work was to determine the morphologic/physiologic changes of the scutellum during germination, using biochemistry, histochemistry and immunohistochemistry techniques and confocal and Normarski microscopy. *Results*. Our results shows the presence of a compartmentalization generated by the fibrous layer, a structure conformed by dead cells from the endosperm with cell walls with impermeable substances. The benefit of compartmentalization is to concentrate and avoid signal and nutriment leaking from the embryonic chamber, allowing an unidirectional mobilization inside the embryo. During postgermination, scutellum epidermis undergoes structural changes in order to become an epithelium, this process is complemented with fibrous layer permeation; all this allows the release of hydrolytic enzymes, and the absorption of substances from the endosperm, then this substances are translocated to the embryo axis in expansion. The transformation of the epidermis into an epithelium involves the lengthening of the isodiametric cells turning into papillate cells; with these changes increases the absorption surface. For cell lengthening cell wall relaxation is required, for this in situ Class III peroxidase acts in a prooxidative way generating reactive oxygen species. Peroxidase is also secreted to the apoplast, and its activity is induced by auxin and nutriments as K^+ or Pi. This correlates with H^+ ATPase activation, apoplast acidification, and changes in oxido-redox status of phenolics in cell walls. *Conclusion*. Maize scutellum have a relevant role: provide substances for the lengthening of the embryonic axis, allows aleurone activation, absorbs nutrients and hydrolytic products derived from the endosperm, and mobilizes metabolites. towards the embryonic axis. All of these functions impact in seed vigor and in seedling establishment with nutritional and economic repercussions.

010. Participation of FIP37 in root development and drought stress in *Arabidopsis thaliana* through the m6A methylation of mRNAs

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mRNA metabolism is regulated by different chemical modifications that may impact stability and degradation. One of these is methylation of the adenosine base at the nitrogen-6 position (m6A) is the most prevalent internal chemical modification conserved among eukaryotic mRNA. In *Arabidopsis thaliana*, m6A is essential for embryogenesis and plant development and is mediated by a multi-protein complex consisting of adenosine methyltransferase A (MTA), methyltransferase B (MTB), FKBP12 interacting protein 37 (FIP37), and others. FIP37 participates in m6A establishment, affecting the metabolism of WUSCHEL (*WUS*) and SHOOTMERISTMLESS (*STM*) that regulate the shoot apical meristem (SAM) development. On the other hand, m6A proved to be a stabilizing label of transcripts under saline stress conditions. We hypothesized that FIP37 could also be involved in root apical meristem (RAM) development and participates in the response to drought stress. Thus, in this study we show the phenotypic and genotypic characterization of a T-DNA insertion mutant in the FIP37 gene (*fip37-p*). We found that the primary root length of the *fip37-p* mutant is shorter than that in Wt. Cellular analysis of this phenotype will be presented. Besides, germination rates under abscisic acid (ABA) treatment indicate that *fip37-p* is less sensitive to this hormone than the Wt. Our results provide a glimpse to the possible role of FIP37 in root development and in the plant response to drought stress.

011. Analysis of microRNAs involved in the vegetative to reproductive phase change in *Agave tequilana*

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During the vegetative to reproductive phase change, shoot meristems become reproductive meristems, leading to flowering. In *Arabidopsis thaliana*, five pathways controlling this phase change have been identified. The aging pathway involves the antagonistic expression of microRNAs miR156 and miR172, that regulate the transcription factors SQUAMOSA PROMOTER BINDING PROTEIN-LIKE and APETALA2 and this pattern is conserved in many plant species. *Agave tequilana* is a perennial, monocarpic plant of great commercial importance for the tequila industry. In this species, the vegetative to reproductive phase change signals the end of the life cycle and readiness for harvesting however heterogeneous flowering and the need to manually remove inflorescences imply higher production costs. A deeper understanding of the regulation of this process will have important applications and help to unravel the differences between *Agave* species and annual or polycarpic species. Transcriptome data suggests that in *A. tequilana*, miR156 and miR172 do not follow the conserved expression pattern, showing an increase in the expression of both miRNAs after the phase change. The purpose of this project is to study the expression of miR156 and miR172 and their putative target genes during different stages of the vegetative to reproductive phase change in *A. tequilana*. Sequences for the precursors for miR156 and miR172 were identified by comparisons to miRbase and their derived secondary structures were shown to meet the current criteria for annotation. Target gene families were also identified and full-length cDNAs were analyzed to locate putative target sequences. Currently *in situ* localization assays are being developed to visualize the accumulation pattern of miR156 and miR172 in shoot apical meristems and leaves of *A. tequilana* plants. Future work will focus on functional analysis by transient expression in a heterologous model and eventually the generation of transformed *A. tequilana* lines.

012. The silencing of fibrillarin in *N. tabacum*

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Fibrillarin is an essential protein for life. It is conserved throughout the evolution and required for multiple functions and is located in the nucleolus and Cajal Bodies (CB). As well, other proteins it is highly dynamic. Fibrillarin participates in various cellular processes such as the Biogenesis ribosomal and its main activity described is as methyltransferase. A known source of methylation for more than 100 methylated sites involved in the early stages of pre ribosomal processing and required for the structural stability of ribosomes. In addition to its function as methyltransferase, it has been associated in viral progression processes. It is possible that the viral proteins interact with fibrillarin is due to the dynamics, between the nucleolus and CB. In previous works in which they have silenced fibrillarin in N. Benthamiana; a change in the phenotype of the plants was observed resulting in “dwarf” plants. Such plants were inoculated with the Groundnut Rosette Virus (GRV), affecting the systemic movement of the virus. In N. tabacum, there are two reported copies of this gene, so in this work the aim is to silence both genes at the same time and individually in order to observe the generated phenotypes and elucidate the functionality of each gene.

013. The nucleolar proteins containing IDR's AtFib, GAR1 and Lsm14 phase separates in vitro

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Most of the principal functions and biochemical processes inside the cell are compartmentalized into organelles, which restricts the principal reactions into a defined volume of the cell. One example of this is the functional nucleolus, an important component of the eukaryotic nucleus, uncharged with the main function of ribosomal biogenesis. Several proteins and other molecules, principally RNA's, proteins and lipids, play significant roles in order to process, modify, and translocate functional ribosomes to the cytoplasm. Some of these proteins exhibit intrinsically disordered regions that are believed to play signatures or binding regions with some RNAs substrates and lipids, but not much is known about their critical function in liquid-liquid phase separations. Two mainly proteins, fibrillarin and GAR1, are the catalytic centers of two different complexes that guide the specific site methylation of ribosomal RNA (rRNA) nucleotide residues and the pseudouridiylation of uracil residues in the rRNA, respectively. Fibrillarin, one of the mayor nucleolar protein components exhibits IDR in its sequence, and in that sense, liquid-liquid phase separations properties. Here we evaluate the *in vitro* capacity of this nuclear protein in order to know if this type of physical separations is possible and the involvement of different partners during this process.

014. The stem cell population are regulated by AtGlsA/ZRF1 in *Arabidopsis thaliana*

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The SAM is formed during embryogenesis and contains a stem cell reservoir that gives rise to almost all aerial part of the plants. Recently we reported an *Arabidopsis thaliana* gene called *gonidialess A* (*AtGlsA*), which is important for maintenance of SAM integrity. We functionally characterized the two genes encoding GlsA orthologs annotated in the *A. thaliana* genome. Double mutants showed stunted growth of aerial and root tissue, the formation of multiple ectopic meristems and effects on cotyledons, leaves, and flowers. Developing embryos in double mutants showed multiple changes in morphology. Some of the embryos showed development of two apical meristems and the planes of the cell divisions were affected. Expression domain of the genes WUS, CLV3, STM were misregulated in double mutants and lack of AtGlsA expression was also associated with changes in localization of auxin and cytokinin. To analyze the transcriptional activity of Wuschel, a chromatin immunoprecipitation assay (Chip-qPCR) was performed using a specific antibody against the trimethylation of lysine 27 of Histone H3 (H3K27me3), and analyzed by qPCR with specific primers for different parts of the WUS promoter. Preliminary results show that in the *atglsa1 atglsa2* double mutant, *WUS* is in a less repressed chromatin state. These results suggest that GlsA is an essential component of the machinery that maintains the integrity of SAM. The next goal is to understand the function of AtGlsA in the maintenance of the Arabidopsis SAM. In order to do this, we will find what proteins physically interact with AtGlsA, to can construct a network in which AtGlsA works. This project will help to know how different plant species develop.

015. Insights on the evolution of plastid differentiation: the role of the GLK transcription factor of *Marchantia polymorpha* in chloroplast development

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Chloroplast development is a complex process that depends on the coordinate expression of nuclear and chloroplast-encoded genes. It is regulated by nuclear and organellar signals capable of modulating gene expression, as well as plant development. Interestingly, some of these signals appear to have evolved prior to the emergence of the Embryophytes clade and could have had a pivotal role on the terrestrialisation; thus, elucidating its evolution may shed light on the latter process. A suitable plant model to address this issue is *Marchantia polymorpha* L., due to its position in an early-divergent clade of the land plants. The GOLDEN2-LIKE (GLK) proteins, members of the GARP family of transcription factors, are positive regulators of chloroplast biogenesis; they bind to the promoters of diverse Photosynthesis-associated nuclear genes (PhANGs) in response to signals derived from the developing plastid. Studies in vascular plants have shown that knockout mutants of these genes exhibit a pale green phenotype derived from defects in chloroplast development. Conversely, their overexpression induces the ectopic differentiation of other types of plastids to chloroplasts in organs such as roots. In this work, we evaluate the function of the GLK transcription factors in *M. polymorpha* and whether their function is conserved. For that, we performed phylogenetic analyses that showed the presence of a single putative orthologue to GLK in *M. polymorpha* (MpGLK). To analyse its function, we generated overexpressing lines of MpGLK, which display a greener phenotype compared to the wild-type plants. Additionally, we generated knockout mutants using the CRISPR/Cas9 technique. Finally, we are producing reporter lines that will allow us to study the expression pattern of the *MpGLK* gene. Our preliminary results suggest that the GLK function might be conserved and, hence, GLK-mediated signalling may predate the origin of land plants.

016. Role of MEDIATOR16 in root development, cell proliferation and auxin response in *Arabidopsis thaliana*

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MEDIATOR (MED) is a conserved multiprotein complex that influences the binding of the RNA polymerase II and the transcription factors to gene promoters. MED has been implicated in several aspects of plant growth and development, defense, non-coding RNA synthesis, maintenance of meristem cell viability and tolerance to biotic and abiotic stress. In *Arabidopsis*, the MEDIATOR16 (MED16) subunit has been involved in flowering phase promotion, iron uptake, and tolerance to cold stress. Here, we show that MED16 is required for the configuration of *Arabidopsis* root architecture. MED16 loss-of-function increases primary root length and lateral root emergence, which correlates with a higher cell division activity in primary and lateral root meristems, without apparent change in auxin accumulation. However, in response to the polar auxin transport inhibitor, N-1-Naphthylphalamic acid (NPA), *med16* seedlings did not experience the expected alterations in root meristem structure, showing longer primary roots than wild-type plants. Detailed analysis of the auxin gene marker *DR5:GFP* showed that neither NPA nor indole-3-acetic acid application alter the auxin response in *med16* root meristems. Thus, MED16 likely orchestrates specific aspects in the regulation of root system architecture through the control of cell proliferation and fine tunes auxin signaling in *Arabidopsis*.

017. Glucose modulates proliferation via TOR in maize during germination

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Seeds need to integrate intrinsic and extrinsic signals during germination for seedling establishment. Among intrinsic signals are simple sugars, which could play a dual role, as a carbon and energy donor as well as signaling molecules.
It has been reported that Glucose (Glc) can stimulate the gene expression of cell cycle positive regulators through activation of TOR kinase in Arabidopsis [(1)]. TOR is a master regulator that coordinates nutrient and energy signaling to promote cell proliferation, among other process. In addition, Glc has a positive effect on cell proliferation at Root Apical Meristems (RAM) in maize embryo axes (MEA) when it is included on imbibition media [(2)].
The objective of this work was to analyze whether the observed Glc effect on cell cycle progression is via TOR kinase activity stimulation, and at what molecular level, in maize under germination.
The strategy was to analyze *de novo* DNA incorporation and the percentage of cells at G1, S and G2/M phases by means of cell DNA ploidy evaluation at RAM in MAE, imbibed on media with/without Glc, including or not AZD8055 (AZD), a specific kinase activity-TOR inhibitor.
The expression of some genes codifying key cell cycle regulatorswas evaluated, the protein abundance of main proliferative modulators, and the size and quantity of cells at RAM.
In order to assess whether AZD was indeed inhibiting kinase TOR activity, S6-phosphorylation, an indirect downstream target of TOR activity, was evaluated in protein extracts from MAE imbibed with Glc or Glc-AZD.
We found out that AZD inhibits TOR kinase activity. Therefore, the results suggest that the via Glu-TOR has a positive role in the control of cell cycle by increasing key genes expression, cell cycle protein markers stability, stimulating *de novo* DNA synthesis and shortening the cell cycle phase timing.

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018. Identification of the presence and activity of possible ionotropic glutamate receptors in *Capsicum annuum* roots through a pharmacological and molecular approach

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The ionotropic glutamate receptors (iGluRs) are integral membrane proteins activated by a signal molecule (agonist) and do they work as non-selective cationic channels (NSCCs) mediating rapid synaptic responses between neurons. Initially, they were discovered in mammals, in which they were classified into three types, according to their pharmacological properties: N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole receptors (AMPA) and kainate (KA). Later, the presence of these proteins was discovered in organisms that did not possess a central nervous system (CNS), like plants. It is now known that they are a family of genes composed of 20 members in *Arabidopsis thaliana*, 24 in *Oryza sativa* and 13 in *Solanum lycopersicum*. In early studies, it has been shown that the functions of these proteins in plants are very diverse, including growth, reproduction and stress response events, among others. Therefore, it is necessary to have a greater number of studies on the same in different plant species, if you want to regulate vital processes of plant biology. To date, there is no knowledge of the presence and function of these proteins in species of the genus *Capsicum.* To analyze its functionality, drugs (agonists and antagonists) regulating the activity of the animals iGluRs are used; however, little is known about their effect on the plant glutamate receptor-like (GLRs) proteins. In this project, these compounds were used to determine the role of these proteins in the radical growth of *Capsicum annuum*. Besides, the presence and activity of possible GLRs in the roots of this species were evaluated using molecular and electrophysiological techniques. Overall, the results indicated that these proteins are involved in the growth and radical proliferation of this type of pepper and that several members of this family can confer great versatility in the response of the roots to different amino acids in the soil.

019. *ALTERED MERISTEM PROGRAM 1* plays a role in seed coat development root growth and post-embryonic epidermal cell elongation in *Arabidopsis*

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Plant development relies on the capacity of cells to interpret positional information and translate it into proliferation, elongation, and differentiation programs. *ALTERED MERISTEM PROGRAM 1 (AMP1)* encodes a putative glutamate carboxypeptidase involved in embryo development, plant growth, and phytohormone homeostasis. Here, we show that mutations of *AMP1* cause defective seed coat formation, which correlates with increased frequency of embryo abortion, low seed production, and retarded germination. Seed alterations in *amp1* mutants were related to decreased production of trichomes on leaves and increased ratio of short or bifurcated root hairs in primary roots and primary root growth inhibition. Expression analyses of hormone-related gene constructs *TCS::GFP*, *DR5:uidA*, and *pABI4:uidA* indicated that slow root growth is likely independent of cytokinin and auxin signaling and involves changes in abscisic acid responsiveness. Our data show that *AMP1* is necessary for normal seed coat and embryo establishment during seed development and plays an important role in post-embryonic root growth and epidermal cell elongation.

020. Molecular cloning and characterization of the *CrPAP1* gene encoding a novel phosphatase like phytase in the green microalga *Chlamydomonas reinhardtii*

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Phytases are a type of phosphatases that hydrolyze phytate, the main source of phosphorus in grains used in animal feed; however, monogastric animals, like swine and poultry, digest poorly the phytate. Phytate has antinutritional effects and when is incompletely digested and excreted to the environment could trigger the eutrophication of water bodies; the phytase addition in animal feed makes possible the phytate digestion and phosphorus disponibility, with positive impact for both the animal industry and the environment. Therefore, phytases are the most commercially important animal feed enzymes.

Chlamydomonas reinhardtii is a photosynthetic microalga that has been used as a model organism for more than 60 years; its nuclear genome has been sequenced and is Generally Considered As Safe (GRAS) by the FDA. *C. reinhardtii* can grow using phytate as sole phosphorus source, but the mechanism of this is unclear. Previously, our research group identified *in silico* six purple acid phosphatase homologues in *C. reinhardtii* (CrPAP1 to 6), where *CrPAP1* gene showed inducibility to phytate, making it the principal candidate for a gene encoding a phosphatase with phytase activity.

In this study, we proposed a new phytase motif based on an *in silico* analysis of several functional phytases and searched for this motif in CrPAPs and found that only CrPAP1 has it, along with a putative secretion motif. We are currently transforming *C. reinhardtii* to express *CrPAP1* under a novel NaCl inducible promoter (*RIA3/PromC*) and a constitutive promoter (*HSP70A/RBCS2*) in order to evaluate the phytase activity of CrPAP1. In addition, we are transforming *C. reinhardtii* with a construct that express the fusion gene *CrPAP1:GFP* to determine the subcellular localization of CrPAP1. To this date, there are no phytases reported from *C. reinhardtii*, therefore this study could be the first report of a phytase from this microalga, and it will expand our knowledge on the phosphorous metabolism in *C. reinhardtii*.

021. The role of BOL an AP2/ERF transcription factor in plant organ development

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Plants are multicellular organisms that possess potential capacity for unlimited growth throughout their life cycle. It is interesting to explore this wonderful plant ability to form organs in a constant way that other organisms do not possess. There are still many questions about the mechanisms involved in organ development. BOL is an AP2/ERF transcription factor of Arabidopsis thaliana that promotes severe alterations in morphology and organ development when is overexpressed. Data from global gene expression analysis using a dominant BOL mutant, revealed expression changes in genes involved in diverse and unexpected cellular processes, showing a complex BOL function. As part of the strategy towards clarifying the BOL function we used a BOL inducible line. We obtained the accumulated transcripts after 30 minutes and 8 hours of BOL induction. From the list of genes that changed their expression at 30 min after BOL induction, we obtained 114 enriched GO terms from the 30 min, and 166 from 8 hours data. One of the most significantly enriched GO categories at 30 minutes is Regulation of Transcription. Most of the genes present in this category encode transcription factors or putative DNA binding proteins. This means that BOL is a master regulator of other transcription factors. From this data we also found that BOL upregulates genes involved in the cell cycle and in DNA replication. The possible explanation that emerges from this, is that, at the very first stages of the development of an organ, BOL pauses the progression of the cell cycle and DNA replication to trigger adjustments in the cell program and thus shape the cell identity.

022. Does paternal genome activation occur in the endosperm of *Arabidopsis thaliana*?

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During early embryogenesis in Arabidopsis, paternal alleles of many genes show reduced activity, consistent with the hypothesis that much of the paternal genome in embryos is silenced for the first few days after fertilization (reviewed in Del Toro-De León et al., 2016). This transient silencing of the paternal genome in the embryo is distinct from imprinting, which has been studied extensively in endosperm development in maize and Arabidopsis. Imprinting in the endosperm affects hundreds of genes, rather than thousands; can result in biased expression of either maternal or paternal alleles; and persists throughout endosperm development. (reviewed in García-Aguilar & Gillmor, 2015). Whether transient silencing of the paternal genome also occurs in the endosperm of Arabidopsis has never been determined.

We are taking different approaches to test the hypothesis that the paternal genome of the endosperm is silenced for the first few days after fertilization. I am performing reciprocal crosses between *emb*/+ mutants and wt plants, to determine if wt paternal alleles can complement mutant maternal *emb* alleles during early endosperm development. I am also using promoter reporter lines to look for differential expression of maternal and paternal alleles in the early endosperm. Preliminary analysis with the *pHISN2::*TdTomato reporter indicates that the maternal allele is expressed at 1 dap, while the paternal allele is not expressed until 3 dap. Finally, we also are planning to profile parent-of-origin contributions to the early endosperm using RNAseq. In addition to its intrinsic interest for studies of gene regulation in plants, my work may also be potentially relevant to agriculture, due to the importance of the endosperm in seed development.

023. Unveiling the Molecular Mechanism of *Arabidopsis* Fruit Dehiscence by Modeling Gene Regulatory Networks

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Fruits, as a seed dispersal units, have evolved different strategies to attain their ecological function, strategies that are also relevant for the preharvest practices. In order to elucidate the molecular basis of the fruit dehiscence, we built a Gene Regulatory Network (GRN) with factors recognized by their central role in the medio-lateral axis formation. Simulating the GRN's dynamics, we were able to recover only three cell types, namely valve, valve margin and replum. This finding demonstrated that the available evidence could not sustain the terminal differentiation of valve margin in lignification and separation layers. We therefore hypothesized that some network components (genes and/or interactions) were missing in this shattering-resistant pod model and, consequently, we decided to assess the role of additional interactions and regulators in the fruit development. According to experimental evidence, these putative regulators might establish genetic interactions with the well-characterized regulators of fruit morphogenesis, although in other developmental processes. After obtaining all the possible cellular phenotypes of each GRN model, we filtered the networks and kept only those that were able to reproduce the cellular patterning observed along the medio-lateral axis: valve, separation layer, lignification layer and replum. Besides, we also simulate the gain- and loss-of-function mutants, and discarded those GRN that did not recover the mutant phenotypes experimentally reported. With this approach, we have been able to propose candidates for the missing components of the dehiscence zone network, and their logical rules of interaction that are able to properly direct the dehiscence zone differentiation. This model constitute a valuable tool to advance our knowledge on Brassica fruit morphogenesis and seed dispersal evolution.

024. Analysis of *AHP6* regulators in *Arabidopsis thaliana* using genome editing

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Fruits produced by flowering plants are important sources of human food, providing nutrition and health. Understanding the regulation of their development can provide valuable knowledge. Fruits originate from the gynoecium, the female sexual organ of the flowers, where fertilization occurs. The gynoecium of the model plant *Arabidopsis thaliana* is formed by two fused carpels. Its development requires a fine and dynamic regulation of genetic and hormonal factors. In this work, we study the control of a negative regulator of cytokinins, *AHP6* which is a pseudo phosphotransferase histidine protein, participating as a negative regulator of cytokinin signaling, and expressed at specific regions of the gynoecium during its development. We postulate that the expression of *AHP6* is controlled by two transcription factors: AGAMOUS (AG), a homeotic gene, key for the identity of the gynoecium and BOLITA (BOL), which participates in the initial stages and but also affects later steps during development. The purpose of this study is to build genetic resources that can provide information about whether AG and BOL are regulators of *AHP6* during the development of *A. thaliana* gynoecium, by either deleting regulatory regions in its promoter or producing strong knock-out mutant alleles using the genome editing system CRISPR-Cas9.

025. Attempting the induction of autonomous embryogenesis in ovules of *Arabidopsis thaliana*.

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Most angiosperms reproduce sexually by generating gametes via meiosis that fuse during fertilization to initiate embryo and seed development. Despite this, sex is not the only reproductive strategy. Some angiosperms have an alternative form of reproduction termed apomixis, which avoids meiosis during gamete formation and leads to the production of clonal embryos derived from the female gamete (gametophytic apomixis) or from sporophytic cells within the ovule (sporophytic apomixis). The mechanisms by which apomictic lines give rise to autonomous embryo development remain unknown. Although the ectopic overexpression of regulatory genes has resulted in the formation of embryos in several vegetative organs, a systematic approach to attempt a deregulation of cell identity that could cause the initiation of embryogenesis in the ovule of Arabidopsis thaliana has yet to be performed. The main objective of this work was to determine the potential of regulatory genes to induce autonomous embryo development by using a set of specific promoters acting in either sporophytic or gametophytic cells of the Arabidopsis ovule: the functional megaspore, the nucellus, and the egg cell. As expected, in some cases ectopic gene expression resulted in aberrant phenotypes during floral development, including changes in organ identity; but they also resulted in developmental aberrations occurred during gamete formation. Interestingly, the ectopic expression of auxin response genes induced the formation of proto-embryonic structures within the ovule as confirmed by the expression of embryo identity markers. We also found phenotypes related to abnormal endosperm formation that could lead to a different interpretation of its function. Our results open new venues for systematically manipulate cell identity in the developing ovule of Arabdiopsis.

026. Mechanisms for megaspore mother cell differentiation in *Arabidopsis thaliana*

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The female gametophyte is essential for perpetuation of flowering plant species. are In most cases the female gametophyte is derived from a single somatic cell that acquires the identity of a pre-meiotic precursor (the megaspore mother cell, or MMC), which goes undergoes meiosis and leads to the formation of a single functional haploid megaspore that divides mitotically to form the female gametophyte. Mutations in Arabidopsis genes acting in the RNA-dependent DNA Methylation (RdDM) lead to the differentiation of ectopic pre-meiotic precursors that can bypass meiosis and form a female gametophyte, a phenotype reminiscent of apospory. In contrast, ectopic precursors in retinoblastoma related1 (rbr1) mutants enter meiosis and form extranumerary haploid female gametophytes. We are conducting a genetic analysis to elucidate possible genetic interactions between the RdDM and RBR1 pathways and determine if both are necessary to avoid the establishment of gametic fate in somatic cells adjacent to the MMC. Segregation distortion and cytological abnormalities suggest possible interactions occurring at the onset of gametogenesis. Our studies constitute a first attempt to establish the genetic basis and molecular mechanisms acting during a molecular cross-talk between RdDM and a circuit controlling cell proliferation through the antagonistic interaction regulating RBR1 activity.

027. *Arabidopsis thaliana* genome edition by the CRISPR/Cas system to elucidate the miRNAs function in male and female organs

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that play crucial regulatory functions in morphogenesis, stress responses, reproductive development and others. In recent years, our research group focus study of miRNAs in sexual plant reproduction using as plant model *Arabidopsis thaliana*. Thus, to elucidate the role of MIR genes in sexual organs by transcriptional fusions of the MIR promoter to uidA gen (GUS) it has been detected that different miRNAs are specifically expressed in female and male reproductive tissues; however, its function is still unknown. This project aims to edit MIR genes that are expressed in reproductive tissue through CRISPR/Cas system to discover their function. Five miRNAs were selected: 159ab, 158, 403, 822a and 867, in all cases the promoter sequence and seed sequence were chosen as independent target to edit. The transgenic lines were obtained by transforming *Agrobacterium tumefaciens* strain GV3101 with the pHSN401 vector, that contains the Cas9 endonuclease under the 35S promoter, and pRI909 vector, with the gRNA under the AtU10 promoter; *A. thaliana* was transformed in isolated events. Once confirmed the presence of the transgene by PCR, the crossing of both lines will have the complete system. To verify a change in the sequence level, the lines obtained through sequencing will be analyzed molecularly. We are currently working on generation of To and T1 mutated lines for later to analyzed phenotypically in sexual tissues. The molecular and cellular analysis will allow us to determine the function of the miRNAs associated with reproductive development.

028. Site of synthesis of ApoCarotenoid Signal 1 (ACS1) in *Arabidopsis thaliana*

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In plants, carotenoids are isoprenoid pigments essential for photosynthesis as well as precursors of phytohormones and signaling molecules. The regulatory function of these compounds during plastid development is not yet understood. The study of carotenoid biosynthesis mutants in the first steps of the pathway, demonstrates that *cis*-carotenoids can accumulate and modulate the nuclear gene expression, resulting in plastid development impairment and variegated or albino plants. One of such albino mutants is the *chloroplast biogenesis 5* (*clb5-1*), that accumulates z-carotene and has finger-like leaves. A double mutant with the *carotenoid cleavage dioxygenase 4* (*ccd4*), restores leaf phenotype, which indicates that a *cis*-carotenoid-derived Apocarotenoid Signal (ACS1) is responsible for the *clb5-1* leaf phenotype. In this work, we wanted to understand whether *cis*-carotenoid derived ACS1 is produced in specialized plastids and tissues, or during specific developmental stages of the Arabidopsis *clb5-1* mutant and wild type (WT). For that, we determined through confocal microscopy, the localization of the PHYTOENE DESATURASE3 (PDS3) and ZETA-CAROTENE DESATURASE (ZDS) enzymes, involved in the synthesis of the *cis*-carotenoid precursors of ACS1, in WT background. To understand the dynamics of the ACS1 synthesis in WT plants, we compared the expression pattern of PDS3 and ZDS to that of CCD4 during embryo development and in early stages after germination *in vitro*. We also explored different growing conditions to comprehend the regulation of PDS3, ZDS and CCD4. In order to gain more information of the site of synthesis of ACS1, we also explored the localization of PDS3 and CCD4 in the shoot apical meristem and finger-like leaves of *clb5-1* albino plants. This strategy will allow us to determine whether ACS1 may be produced in specific tissues of green plants or under specific growing conditions.

029. *NaTrxh activates S-RNase for pollen rejection in Nicotiana.*

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Many plant species have self-incompatibility (SI) systems that enhance outcrossing by discriminating between "self" and "non-self" pollen and specifically rejecting self-pollen. *Solanaceae* SI response depends on S-RNase and SLF/SFB *S*-specific interactions. *S*-RNase is a cytotoxin that acts against self-pollen degrading its RNA and inhibiting its growth. Besides, other factors are required to pollen rejection response, named Modifier Genes (MG). Here, we studied *NaTrxh* as a possible MG in *Nicotiana* and its possible biochemical mechanism in SI.
NaTrxh is a type *h* thioredoxin with higher expression in pistils of SI *N. alata* than in those of self-compatible species. Previous reports reveal that NaTrxh is secreted onto the pistil extracellular matrix, that it interacts with the S-RNase, and reduces it *in vitro*.
Here, we show that expressing a redox-inactive mutant, $NaTrxh_{SS}$ in transgenic hybrids, behaves as a dominant-negative that suppresses *S*-specific pollen rejection. To get further insight, we performed biochemical assays and found that NaTrxh specifically reduces the Cys_{155}-Cys_{185} disulfide bond in the S_{C10}-RNase increasing its ribonuclease activity seven-fold. With this information, we give shed light into the redox regulation in SI mechanisms and we propose that reduction and activation of S-RNase by NaTrxh helps to explain why S-RNase alone is not sufficient for pollen rejection and why it is cytotoxic only against the self-pollen.
In addition, we tested whether the N- or C-terminal domains in NaTrxh are involved in the S-RNase activation by creating mutant proteins lacking each domain; our results show that the N-terminal domain is involved in this reduction and activation. Because the N-terminal interaction is crucial for SI, we are working in molecular dynamic simulations between the crystal structure of NaTrxh that we have recently determined at 1.7Å and the previously reported S_{F11}-RNase.

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030. miRNA regulation of egg and zygote polarity

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The egg cell of Arabidopsis is polarized, with an apical nucleus and a basal vacuole. This polarity is lost after fertilization, and then reestablished in the late zygote (Kimata et al., 2016). The first division of the zygote is asymmetric, resulting in a small apical cell that will produce the shoot apical meristem, cotyledons, vascular tissue, and the epidermis; and a large basal cell that produces the root meristem and the suspensor.
microRNAs (miRNAs) are a class of small RNAs that regulate mRNA stability and translation, and play important roles in plant and animal development. Our laboratory has recently shown that miRNAs are required for the apical-basal polarity of the zygote, and for its asymmetric division. Loss of the enzymes DICER LIKE 1 and SERRATE, which are required for processing miRNA precursors into mature miRNAs, result in zygotes that lack correct polarity of the nucleus and vacuole, and divide symmetrically (Armenta-Medina et al., 2017). This symmetric division of the zygote results in embryos with a duplication of cell fates, and which arrest several days after fertilization (Schwartz et al., 1996; Lobbes et al., 2006; Nodine and Bartel., 2010; Seefried et al., 2014).
The objectives of my project are to determine whether the altered polarity of the zygote in *dcl1* and *se* mutants originates in the egg cell, and to characterize the effect of loss of miRNA function in the egg and zygote, using molecular markers for cellular compartments and cell identity. The results of these experiments will provide important information on the role of miRNAs in establishing the first two cell lineages in plant embryogenesis.

EPIGENETICS

031. *ARABIDOPSIS HOMOLOG OF TRITHORAX* (*ATX1*) and its target genes participate in root development

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Chromatin state changes are crucial for plant development. The *ARABIDOPSIS HOMOLOG OF TRITHORAX (ATX1)* gene encodes a histone methyltransferase directly involved in the trimethylation of the lysine4 (H3K4me3), which is associated with open chromatin and active transcription. ATX1 is important for cell cycle duration, cell production, stem cell niche maintenance, and cell patterning during root development (Napsucialy-Mendivil, *et al*. 2014).To identify candidate genes regulated by ATX1 and involved in root development, we performed transcriptome sequencing (RNA-Seq) of the *atx1-1*^{setm} mutant root tissues. In this mutant, the ATX1 protein maintains its structural integrity but it is catalytically inactive. The mRNA was extracted from roots of 10-days after germination seedlings, in two independent experiments. The transcriptome profiling of the *atx1-1*^{setm} root showed that 355 genes were differentially expressed (195 downregulated and 160 upregulated, fold change ≥2). The RNA-seq data were validated by RT-qPCR analysis of transcript abundance of selected genes. Remarkably, several putative targets of ATX1 were identified: *TEOSINTE BRANCHED/ CYCLOIDEA/ PROLIFERATING CELL FACTOR2 and 24*, *AGAMOUS LIKE14*, *LATERAL ORGAN BOUNDARIES DOMAIN*29, and *HOMEOBOX PROTEIN21* transcription factors were downregulated in the *atx1-1*^{setm} background. Also, some pericycle-specific genes were differentially expressed in the *atx1-1*^{setm} background; one of them, the *PEROXIDASE 35-RELATED,* was downregulated. Interestingly, the characterization of the root development in the *peroxidase 35-related* mutant showed that while primary root growth was not affected, the lateral root primordium morphogenesis was abnormal and had a phenotype similar to that found in *atx1-1*^{setm} mutant. These results suggest that *PEROXIDASE 35-RELATED* might be directly regulated by ATX1.

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032. *mncEIN2* is a non-coding intermediate RNA with important role in the ethylene perception pathway.

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Intermediate non-coding RNAs (*ImncRNAs*) are molecules with regulatory function, and a length between 50 and 300 nucleotides. In the plant model, *Arabidopsis thaliana*, there is limited information about *ImncRNAs* and their function. However, it is known that they are regulated by genetic and epigenetic mechanisms similar to coding proteins genes. In addition, it has been characterized that the low regulation of several *ImncRNAs* results in molecular changes and abnormal development phenotypes in plant growth and development, suggesting an important role in such processes. In the present study, we show that *ImncEIN2* is located downstream of the *EIN2* gene, its characterization, and its possible participation in the ethylene perception pathway. The ethylene perception pathway is activated in several development plant processes, and it has been elucidated through the characterization of mutant plants that show an altered phenotype of the triple response. The ethylene perception begins in the membrane of the endoplasmic reticulum and transduces the signal to the nucleus with the activation of a transcriptional cascade that triggers the activation of several ethylene-responsive genes. In this perception signal transduction pathway, EIN2 plays a central role. The mutant phenotype of *ImncEIN2* reiterates its participation in the ethylene pathway, showing an altered phenotype in the triple response as well as the deregulation of genes-related to the ethylene response. On the other hand, we found a synteny between the genes of *EIN2* and *ImncEIN2* as well as the possible formation of secondary structures present in the *ImncEIN2*, which is conserved in other organisms suggesting a possible conservation of its function.

033. A long intergenic non-coding RNA is a novel positive regulator of the ABI FIVE- BINDING PROTEIN (*AFP1*) in *Arabidopsis thaliana*

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Noncoding RNAs (ncRNAs) have emerged as major products of the eukaryotic transcriptome with regulatory importance. In *Arabidopsis*, long ncRNAs (lncRNA) are expressed at lower levels than protein-coding genes, but exhibit tissue-specific and stress-responsive expression patterns, therefore, the effort in the characterization of its functions has grown, focused on the elucidation of its participation in the mechanisms developed by the plants, to combat environmental stresses. The phytohormone abscisic acid (ABA) plays a vital role in inhibiting seed germination and in postgermination seedling establishment. In the ABA signaling pathway, ABI5, a basic Leu zipper transcription factor, has important functions in the regulation of seed germination. ABI5 protein localizes in nuclear bodies, along with AFP1 (ABI Five Binding Protein 1) and, this protein attenuates ABA signals by targeting ABI5 for ubiquitin-mediated degradation in nuclear bodies. Recently, an intergenic lncRNA has been identified within the *afp1 locus*, hence we named as *lincAFP1*, which it is expressed under abiotic stress conditions; however, its function is unknown. In this study, we show that overexpression and deficiency of *lincAFP1*, increases and decreases, respectively, the expression of its adjacent genes (*afp1*, *ntf2*). In addition, plants with a deficiency of this gene showed a hypersensitive phenotype with a delay in the germination under the ABA treatments. Meanwhile, the overexpression of the gene results in plants with an insensitive phenotype to ABA treatment. Our results suggest that, *lincAFP1* acts as a positive regulator of the afp1 expression and therefore stimulates the tolerance to ABA stress and it is important to determine the mechanisms of how the regulation is carried out suggesting that could be due to the recruitment of chromatin modifying complexes enabling the transcription of *afp1*.

034. *lncTATA* a long non-coding RNA involved in leaf development of Arabidopsis thaliana

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The long non coding RNAs (lncRNAs) are molecules with a length greater than 300 nucleotides that regulates the expression of multiple genes involved in important processes. In plants some lncRNAs have been described as regulators of the flowering time, as well as modulators of the expression of genes involved in the response to biotic and abiotic stresses. The characterization of their functions have been a major interest for several groups, due to the grand diversity of functions, structures in to the large numbers of biological processes where they are participating. This project shows the characterization of an lncRNA in *Arabidopsis thaliana*, which is classified as a promoter lncRNA of the gene *TBP2* (*lncTATA*). TBP2 is an important member of the transcription pre-initiation complex, which also interacts with the machinery of miRNAs transcription. We analyzed the relative expression of TBP2 in mutant lines and overexpressing lines (OE) of *lncTATA* and we found a down and up regulation in mutants and OE lines respectively, these results suggest that *lncTATA* could be a negative regulator of TBP2. On the other hand, the *lncTATA* mutant phenotype showed a decrease in area and the serration of the leaf. In addition, the expression analysis of genes involved in leaf development and maturation such as GRF and SPL, as well as some miRNAs (miR396, miR172 and miR156) showed a deregulation. This evidence showed us that *lncTATA* negatively regulates the expression of TBP2 altering miRNA accumulation causing then a deregulation in the development of *A. thaliana* leaves.

035. Effect of Hydrogen Peroxide in the Global Methylation in *N. tabacum*

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The hydrogen peroxide (H_2O_2) plays a principal role in the biotic and abiotic stress responses in the plant, due to H_2O_2 is one of the firsts compounds generated after a stimulus, and triggered a complex signal transduction network to modulate response. It has been observed that treatment of H_2O_2 can improve the tolerance state of the plant to several stress conditions. Recently, it has been suggested that the H_2O_2 may have influence in the epigenetic gene regulation, thus it is possible that the H_2O_2 could be a potential elicitor for the stress memory in plants. To determinate the effect of the H_2O_2 in the global methylation of the plant, we tested it with two groups of plants: 1) Two transgenic plants: a line with a continuous H_2O_2 production (L8) by the insertion of *CchGLP* gene codifying a Mn-SOD, and an azygotic line (L1) which have the gene inserted but it's not expressed; 2) Wild type plants: a group of plants elicitated three times with H_2O_2 200 mM and a group of control plants not elicitated. At the six weeks and the twelve-week, samples of leaves and roots were taken from all the plants. These were used by DNA extraction by the CTAB protocol, and then in the 5-mC DNA Elisa kit. The results displayed that, the L8 transgenic plants had hypomethylation in comparison with the L1 in leaves and roots at both time sampling. By the contrary, the elicited plants presented hypermethylation compared with the control. In both groups of plants, there were no significant differences in morphological features as height, fresh weight, stem width and chlorophyll content; just a slight difference was observed in leaf area. These data suggest a high expression of genes in the H_2O_2 high producer transgenic plants, which corresponded with another "omic" data of the model and its phenotype. Interestingly, in the plants treated with H_2O_2, it seemed that high gene repression it has been induced, but without affecting the morphological aspects of the plants.

PLANT GENETIC IMPROVEMENT

036. Somaclonal variation analisys in *A. angustifolia* (espadin) based on molecular markers

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Tissue culture can be used to vegetatively propagate elite material or to generate new variability by employing somaclonal variation (SV). This is the term used to define the variability generated by tissue culture, and is the combination of genetic and epigenetic variation. Extensive work has been carried out in the study of SV in both monocotyledonous and dicotyledonous species; at present the evidence suggests that the genotype used is one of the major factors involved in SV. In this work, Amplified Fragment Length Polymorphism, (AFLP), was used to evaluate the stability of DNA in regenerated plantlets of *Agave angustifolia* (espadin) used to produce mescal, and collected on the Oaxaca and Guerrero states. A total of six primer combinations was used and the similarity index was obtained using DICE coefficient. We observed: i) a low genetic variation comparing the in vitro plants, after more than 10 cycles of subculture, with the material used to induce the tissue culture; ii) genetic differences comparing the same species from Guerrero and Oaxaca, which suggest natural genetic variability across the distribution area on this species. This work is the base to guaranty the genetic stability during scaled up propagation of “agaves mezcaleros” to support the industry of mescal.
The authors are grateful to the grant “Programa de Estancias Posdoctorales para Mujeres Indígenas en Ciencia, Tecnología, Ingenierías y Matemáticas” from IDRC-CONACYT-CIESAS (C-873/2018).

037. Mining genomic data to identify novel proteases s8 in plants

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The global market for commercial enzymes is about $4.4 billion a year, and proteases are the most important type of industrial enzymes as they represent 60% of all commercialized enzymes in the world. Plant proteases have been used in medicine, detergent manufacturing, and food. However, their extraction from plants is decreasing because it is more expensive compared to those of microbial origin. However, DNA technology and genetic engineering play an important role in the identification and production of these proteases thus, novel proteases with attractive biotechnological properties for the industry are emerging trough these forefront methods.
Within proteases, S8 family, subtilases or serine proteases are one of the best-characterized groups of proteolytic enzymes in higher organisms and among proteases, they represent 40% of the total global enzymes for industry. Proteases S8 are characterized by having a catalytic triad of three amino acids: aspartate, histidine and serine, and are abundant in plants, for example, 63 genes are known in Oryza sativa genome and 56 in Arabidopsis thaliana. In this study, we have developed a faster and efficient method of data extraction to identify proteases S8 in plants genomic data. Our method includes: (i) Designing of regular expressions using the sequences of proteases S8 of CDD from NCBI and MEROPS database, (ii) obtaining plant proteases S8 sequences using the customized regular expression, and (iii) corroborating the proteases S8 new sequences by aligning the catalytic triad region of each sequence to the S8 family of MEROPS database. Our bioinformatic pipeline was able to recover 67 proteases S8 sequences from A. thaliana genomic data; 45 out of 63 proteases S8 reported for O. sativa; and 43 proteases S8 sequences from the MEROPS database. These results suggest that our method to identify proteases S8 is efficient, robust, cheap and can be applied to several plants.

038. A study of genetic diversity of the native maize Jala landraces, using SNPs mapped on the maize reference genome (B73, v.4)

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Currently, the techniques related to the improvement of maize (*Zea mays* L.) have gained great importance in Mexico. In this sense, the application of molecular markers has increased the efficiency in the identification and selection of characteristics with agronomic interest. For this reason, it is considered that Principal Component Analysis (PCA) represents a complementary statistical technique that allows the exploration of a large amount of data, in order to predict models. Therefore, the present project had as a main objective to analyze and classify the genetic diversity of the Jala maize landrace, through the PCA using Single Nucleotide Polymorphisms (SNPs) markers mapped on the maize reference genome.
In this study, we analyzed 39,986 SNPs generated by DArTseq Technology; markers were developed in the Genetic Analysis Service for Agriculture (SAGA) laboratory at CIMMYT. From the selected SNPs, 58% (23,197), were distributed in the 10 chromosomes, while the rest (16,789) were located in an unmapped region, according with the maize genome reference (B73 v.4; http://www.maizegdb.org/assembly). The SNPs were discovered from 60 Jala accessions, corresponding to five different regions: Nayarit, Colima, Jalisco, Chihuahua and Guatemala.
A PCA was carried out using three components to select the samples that contributed, to a greater extend, to the genetic variation. The graphical analysis was made with the R statistical software package, and PCA analysis were performed on the 10 chromosomes. The results showed for 10 chromosomes two large independent groups, corresponding to the accessions collected at Nayarit state, and another group that represents Jalisco. Separated groups corresponding to the following accessions were also identified; NAYA_54, NAYA_340, GUAT_418, CHIS_94, GUAT_499, COLI_22 y GUAT_529. Therefore, we could say that the largest output variations found in the different populations of Guanajuato, Nayarit and Colima may be due to various aspects. This kind of studies could be used as a tool to design conservation strategies or use the information in plant breeding programs.

039. Genetic analyses of micropropagated achiote (*Bixa orellana* L.) plants

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Bixa orellana L. is characterized as the main natural source of bixin, a red-colored dye found in its seeds; bixin has a high potential commercial use in the food, pharmaceutical, and cosmetics industries. However, bixin contents vary considerably in *Bixa orellana* L. plants because of its phenotypic heterogeneity, poor germination, low viability of its seeds and limitation for its vegetative propagation via stem cuttings or layers which interferes with the establishment of homogeneous commercial plantations, hampering bixin production. To date no agronomic varieties have been registered and the plant has been characterized only by the variability of its forms (flower and fruit) and the levels of bixin content (morphotypes). A micropropagation protocol was established in vitro from hypocotyl explants of one seed germinated in vitro aiming to solve the problem of plantations heterogeneity by obtaining genetically homogeneous plants. Four achiote morphotypes clones with high bixin contents were obtained. The use of sequence related amplified polymorphism (SRAPs) and Simple Sequence Repeat (SSR) molecular markers will establish the basis to learn if changes in the genetic structure of the micropropagated individuals occurred. If we can find no genetic changes in the micropropagated morphotypes, they could be used as parental plants to produce agronomic morphotypes with greater bixin contents.
Keywords: Micropropagation, clones, SRAP, SSR.

040. Application of the intragenic improvement method in a maize genotype of the Mexican central highlands

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Genetic breeding aims the modification of target genes for conferring agronomic advantage to crops. It relies on the use of marker genes that show a positive correlation with quantitative traits. On this regard, we are using the information of conventionally maize breeding previously attained, to select marker genes: rubisco activase (RCA, related to increasing the photosynthetic rate) and dehydrin Rab17 (related to drought tolerance). These genes were incorporated in intragenic molecular constructions designed to overexpress each gene. These constructions contained exclusively DNA sequences from the maize genome, without any selection genes or DNA sequences from virus or bacteria. Maize embryogenic calli (E) of the Tuxpeño VS535 variety, were transformed with the intragenic constructions, and the regenerated plants successfully overexpressed each protein. These results show that the intragenic method could be an alternative technique for crop improvement, which could be applied to other genotypes. In this work we explore the potential use of the intragenic method for transforming maize genotypes developed at the Colegio de Postgraduados (COLPOS, central Mexico). The embryogenic capacity of five selected genotypes was evaluated using immature embryos to obtain calli. Two genotypes regenerated plants from E callus, but only the genotype P-67 produced a high number of plants. This genotype was then transformed with the intragenic constructions that overexpressed constitutively RCA and conditionally Rab17, with the biolistic. Then plants were regenerated from E calli and acclimated to grow under greenhouse conditions. The transformed plants that incorporated both constructions were identified by PCR and specific primers. Protein expression was evaluated by WB and specific antibodies. Results showed that the method can be applied to embryogenic maize genotypes, although further adjustments are still necessary.
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041. Genetic diversity study on Jala maize landrace (*Zea mays* L.), using a principal components analysis (PCA)

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Maize is a crop very famous due its wide genetic diversity, and Jala is appreciated for its particular phenotypic characteristics. The constant recombination between the components of this landrace, as well as the environmental pressure, has caused changes in the expression of its phenotype. The main objective of this study was to identify the genetic variants of 60 Jala accessions corresponding to five different locations, in order to recognize de genetic distances between individuals belong to part of the Jala collection protected in the CIMMYT international germplasm bank. We identified 75,662 good quality Single Nucleotide Polymorphism (SNP), these molecular markers were developed using DArTseq technology; and performed by the Genetic Analysis Service for Agriculture (SAGA) lab, which is located at CIMMYT in Mexico. Subsequently, the diversity analysis was carried out by constructing a population data matrix, with the allelic frequencies calculated for the 60 accessions. After data depuration by discarding the SNPs located in the Minor Allelic Frequency (MAF) <0.05 and > 0.95; 39,986 SNPs were retained and developed a Principal Components Analysis (PCA) using the R Statistical software developed by the Biometrics and Statistics Unit from CIMMYT. The results showed three compact groups, in which the accessions collected at the state of Nayarit and Jalisco are concentrated in the first group. In the second group, although the phenotypic characteristics are different, the analysis that blend the genetic information shows that different Jala accessions from Nayarit maintained genetic variations (NAYA 202, 302, 204, 294), including some accession of Chiapas. In the third group, the accessions from Guatemala also showed a separate core. It is concluded that the accessions of Nayarit as well as Jalisco show the least genetic variation, since they are in a single compact group, while that anyone accessions of Nayarit show genetic variation,

042. Metabolic engineering of *Synechococcus elongatus* to control biological contamination in raceway ponds

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Open pond cultivation (mainly raceway ponds) is the most promising and environmentally friendly system for the production of microalgae and cyanobacteria biomass as it requires less energy and their construction and operation is easier and less expensive than the other systems. However, although open raceway ponds allow the use of sunlight, they are highly exposed to biological contamination. To face this problem, species capable of tolerating extreme environmental conditions have been used (e.g. *Arthospira platensis*) which significantly reduces the risk of contamination by weedy organisms. However, because this strategy prevents the use of most cyanobacteria or microalgae species, treatments with chemicals, biological and physical agents are implemented for the cultivation of those unable to grow under extreme conditions. Although the use of these compounds is relatively effective, antibiotics and herbicides are currently not well accepted by the consumer and increase also production costs. Recently, we reported a strategy based in metabolic engineering to effectively control biological contaminations during *Chlamydomonas reinhardtii* cultivation. This technology is based on the expression of the ptxD gene that encodes a phosphite oxidoreductase which converts phosphite (Phi), a non-metabolizable compound, into phosphate, allowing the establishment of a highly selective cultivation system. Here, we report the successful implementation of this system in the cyanobacteria *Synechococcus elongatus*. We demonstrated the correct integration and expression of the ptxD gene in transgenic lines, which were capable or growing on media with Phi as the sole phosphorus (P) source and becoming the dominant species in mixed cultures. We established large-scale cultivation in raceway ponds with non-sterile growth media with Phi as the sole P source, and were able to effectively control contaminations. Therefore, our system will contribute to avoid sterilization of media and bioreactors, to decrease operation costs and make possible the use of a wide range of cyanobacteria species for diverse purposes.

043. Generation of protocols for the genetic transformation of ornamental plants of Haworthia sp.

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The genus *Haworthia* are succulent plants that are grown for ornamental purposes as they are characterized by having special characteristics for growers. The requirement of collectors is increasing, its demand lies in the uniformity of plants and attractive physiological properties. Obtaining new varieties takes a long time, therefore, in this work we propose the cultivation of plant tissues and genetic transformation for obtains *Haworthia* plants with new characteristics. In the present work we study the species *H. truncata, H. maughanii, H. camptoniana* and *H. retusa*. Green callus friable for Haworthia species were obtained in a basal medium MS 0.5X (Murashige & Skoog) supplemented with Thidiazuron (TDZ) 1 mg/L and Naphthalenacetic acid (ANA) 1 mg/L. For to evaluate the sensitive of calluses to the section markers, they were seeded in MS 0.5X medium with hygromycin (0, 100, 150, 200, 250 mg/L) or BASTA® herbicide (0, 150, 200, 250 y 300 mg/L). *H. retusa* calluses died 31 days after sowing on MS 0.5x with Hygromycin 150 mg/L. For *H. truncata*, it was observed that calluses showed a cream color and decreased their differentiation rate 18 days after sowing in hygromycin 250 mg/L. In the case of the *H. maughanii* and *H. camptoniana* species for day 38 after planting, both species showed a 90% loss of color in their calluses. Currently, sensitivity curves using BASTA® are carried out. In addition, we development an *Agrobacterium*-mediated transformation protocol for *Haworthia* calluses that currently is being evaluated to determinates transformation efficiency.

044. Biotechnology in clonal propagation and genetic improvement of pineapple (*Ananas comosus* (L.) Merr.).

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Pineapple (*Ananas comosus* (L.) Merr.) it is the most economically important species of the Bromeliaceae family. It is a tropical fruit grown for food in productive systems, which has a significant commercial value due to its pleasant taste and aroma, as well as its high content of vitamins and minerals that have made it a highly demanded fruit worldwide. However, it has numerous limitations for its production on a commercial scale due to the low availability of quality propagules and their genetic heterogeneity that limit pineapple production for the national and international market. Taking into account that the variation that is generated during the culture of somatic cell tissues is a valuable tool in the genetic improvement of plants, the objective of this work is to develop a biotechnological system that allows to overcome these problems through clonal propagation and the selection of promising genetic materials generated from in vitro callus. Molecular markers will be used to evaluate the genetic stability and the possible induced variation in the different protocols developed and for this, direct regeneration systems (multiple outbreaks) and indirect regeneration (callus and neo-formations) were established from different types of explants of the cultivars 'Cayena Lisa' and 'MD2'. Advances are described at work.
Key words: Pineapple, clonal propagation, somaclonal variation.

045. Optimization of polymerase chain reaction based on response surface methodology for the detection of gene I-3 associated to the resistance of tomato to the wilting or fusariosis

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INTRODUCTION. Mexico is one of the largest tomatoes (*Solanum lycopersicum* L.) producers in the world, in which Sinaloa contributes about 28% of annual production. However, its production has been affected by various diseases, such as; the wilting also called fusariosis caused by the fungus *Fusarium oxysporum f. sp. lycopersici* (Fol) which affect the tomato root and causes serious economics losses in the crop. The chemical treatment of this disease has caused a negative impact on the environment and promoted the emergence of resistant pathogens. One of the strategies to cope against Fol is the use of resistant varieties, which can be selected by detection of molecular markers. The aim of this work was to obtain the optimal conditions for the endpoint PCR amplification of the molecular markers associated to Fol resistance in commercial tomato varieties by means of a response surface method (RSM). MATERIALS AND METHODS. The commercial Fol-resistant hybrid SV8444TE and the susceptible cultivar DRD600, which are regularly sown in Sinaloa, were used for endpoint PCR optimization. DNA was extracted from 0.3 g of fresh leaves using the 2.5 % CTAB method. For RSM, a central composite design for four factors (Annealing temperature (Ta), DNA, MgCl2 and primer concentration) with 30 trials was used to determine the interaction between factors. The fluorescence intensity from the amplified bands was considered as the response variable and was analyzed for RSM using MINITAB 18.1. The PCR products were run in a 1% agarose gel, stained with ethidium bromide and visualized with UV light. RESULTS. The optimal conditions for the amplification of I-3 were: Ta 63°C, DNA 32 ng, MgCl2 1.5 mM and primer 0.5 µM, with composite desirability of 0.99. CONCLUSIONS. The reported results in this research demonstrate the feasibility of RSM as an optimization tool for methods based on endpoint PCR.
Keywords: Tomato, PCR, I-3

046. Optimization of polymerase chain reaction based on response surface methodology for the detection of Ty-1, Ty-2 and Ty-3 genes associated to the resistance of tomato to tomato yellow leaf curl virus

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INTRODUCTION. Tomato (*Solanum lycopersicum* L.) is one of the main export vegetables grown in Mexico, in which Sinaloa contributes about 28% of annual production. However, tomato production has been affected by various diseases, such as TYLCV, which causes the greatest devastation in the crop. The chemical treatment of this disease has caused a negative impact on the environment and promoted the emergence of resistant pathogens. One of the strategies to cope against TYLCV is the use of resistant varieties, which can be selected by detection of molecular markers. The aim of this work was to obtain the optimal conditions for the endpoint PCR amplification of the Ty-1, Ty-2 and Ty-3 molecular markers associated to TYLCV resistance in commercial tomato varieties by means of a response surface method (RSM). MATERIALS AND METHODS. The commercial TYLCV-resistant hybrid SV8444TE and the susceptible cultivar DRD600, which are regularly sown in Sinaloa, were used for endpoint PCR optimization. DNA was extracted from 0.3 g of fresh leaves using the CTAB method. For RSM, a central composite design for 4 factors (Annealing temperature (Ta), DNA concentration, MgCl2 and primer) with 30 trials was used to determine the interaction between factors. The fluorescence intensity from the amplified bands was considered as the response variable and was analyzed for RSM using MINITAB 18.1. The PCR products were run in a 1% agarose gel, stained with ethidium bromide and visualized with UV light. RESULTS. The optimal conditions for the amplification of Ty-1 were: Ta 60°C, DNA 90 ng, MgCl2 3.36 mM and primer 0.13 µM. For Ty-2, Ta 54.4°C, DNA 10 ng, MgCl2 1.5 mM and primer 0.9 µM. For Ty-3, Ta 45.6°C, DNA 90 ng, MgCl2 3.5 mM and primer 0.1 µM; all of them with composite desirability of 1. CONCLUSIONS. The reported results in this research demonstrate the feasibility of RSM as an optimization tool for methods based on endpoint PCR. Keywords: Tomato, PCR, TYLCV.

047. Somatic embryogenic callus induction in *Sansevieria trisfasciata*, an emerging model for fiber functional genomics in monocots

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Fibers are specific cells than are present in many different plant tissues and usually provide mechanical support. Leaf fibers occurring in bundles (flax, jute, sisal) and those ones found in fibrovascular woody tissues (wood fibers) have a world commercial importance. With an exception of *Gossypium hirsutum* (ingle-celled extensions of the seed epidermis) and *Arabidopsis thaliana* (interfascicular and xylary fibers), genomics underlying differentiation and development of fiber is little known in plants. *Sansevieria trisfasciata* is a tropical CAM monocot with a high leaf fiber content, and easily cultivated under extreme environment conditions (drought, high temperature). Sansevieria fibers runs longitudinally along the leaf and can be easly extracted. Here, we report for the first time an efficient protocol for embryogenic callus induction in *S. trifasciata* cv. Hanhii and cv. Laurentii, two cultivars with contrasting fiber contents. Additionally, a novel expression binary vector for monocots, with a double reporter gene is also described. Our results constitute a value platform for future works focused on functional genomics of plant bundle fibers.

048. Cloning of the DLO1 susceptibility gene in banana and assessing its role in disease resistance using CRISPR/Cas9

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Banana is the most popular fruit in the world and a key element in terms of food security in developing countries, providing a cheap and easily source of energy and nutritional benefits. Like many other crops, banana is threatened by biotic and abiotic factors. As part of the biotic factors related to banana cultivation, *Pseudocercospora fijensis* and *Fusarium oxysporum*, the causal agents of Black Sigatoka disease and Panama disease, respectively, are responsible for significant losses every year. *P. fijiensis* requires large amounts of fungicides for its control, up to 66 applications (US $1000/ha) per year which is very expensive and to the detriment of the environment. *Fusarium oxysprum f.sp*. *cubense* tropical race 4 (TR4), a soil-borne fungus is even more lethal since the fungicides are inefficient to control it, so the banana production as we know it, is at risk of disappear. The above leads us to the need to propose new strategies to develop banana plants with resistance to these diseases taking into consideration environmental sustainability, reduction of carbon footprint, human health, and food security. Different strategies are focused on the introduction of resistance genes in susceptible plants. On the other hand, susceptibility genes are involved in the suppression of the resistance mechanisms of the plant host cell. The Arabidopsis Downy Mildew Resistance 6 (*DMR6*) genes and its homolog DMR-like 1 (*DLO1*) are involved in the suppression of resistance against different hemibiotrophic fungi and is considered as an interesting disease susceptibility gene to investigate in crops such as banana. The use of novel biotechnology tools for genome edition, as CRISPR/Cas9 system, opens a wide range of possibilities to genetically improve crops. The aim of this work, is the generation of *dlo1* mutant plants of banana using CRISPR/Cas9 and evaluate their resistance against different banana pathogens.

049. Characterization morphological and molecular diversity in collection bank and cultivated of achiote (*Bixa orellana* L.) from Yucatan

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Bixa orellana is a tropical plant used for the natural pigments present in its seeds. This plant is the only cultivated species of the genus Bixaceae and is known as achiote in México. Nevertheless, cultivation and hybridization within the species present large morphological variations and variations in pigment content between different cultivation systems. Our principal aim was therefore to analyze variability within the different cultivation systems. Agronomic and genetic traits were used to identify genetic material of agronomic importance that is molecularly associated with polymorphisms of microsatellite regions in order to develop a more adequate selection procedure in the species. Principal components analysis (PCA) and hierarchical cluster analysis (HCA) were the multivariate tools used to obtain the different morphogenetic and molecular variables. We concluded that the combined analysis of morphogenetic and molecular traits represents the optimal approach for characterizing and evaluating variability and correlating it with agronomic traits that allow obtain elite plants that serve as a genetic basis for the study of bixin synthesis, the main commercial compound, whose route remains poorly elucidated as well as base to genetic improvement to be carried out on the compound or trait that it is sought to elite plants and those that are useful for generating commercial agronomic varieties.
Keywords: Achiote, Natural pigments, Genetic variation, PCA, HCA.

050. Genetic diversity analysis of the maize Jala landrace

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At the international level, Mexico is considered within a group of countries in the top ten producers of maize (*Zea mays* L.). One of the most traditional landraces is called Jala, it has a particular phenotype, taking more relevance its ears size. The present project focuses on characterizing the genetic diversity of part of the Jala collection protected in the CIMMYT international germplasm bank, using molecular markers such as Single Nucleotide Polymorphism (SNP). To achieve this goal, 75,662 SNPs have been identified using DArTseq technology, which were developed by the Genetic Analysis Service for Agriculture (SAGA) laboratory located at CIMMYT. The molecular markers were identified from 60 Jala accessions, collected in five different locations: Nayarit, Colima, Jalisco, Chihuahua and Guatemala.
In order to improve the quality of the results, the parameter of Minor Allele Frequency (MAF) was performed by selecting the markers within >0.05 and <0.95 range. After the filtering process, 39,986 SNPs have been selected, from which 23,197 SNPs were distributed on the 10 maize chromosomes. The results of the diversity analysis, obtained by using the BIO-R software, showed a Shannon index of 0.763182323 with a standard deviation of 0.00147342. The Shannon index is one of the diversity measures, it is equivalent to the level of uncertainty in regards to the identity of an element taken at random from a collection of “N” elements distributed in “S” categories. In the dendrogram generated by Archaeopterix, three isolated materials were identified with the highest diversity indexes corresponding to the following accessions: NAYA_54 (0.999249057), GUAT_418 (0.99915532) and NAYA_340 (0.998019889).
In conclusion, the results showed that in the Mexican state Nayarit, as well as in a nearby country as Guatemala, there is a considerable genetic diversity on the Jala landrace. This diversity could be used in maize breeding programs. Markers obtained from diversity, but related with specific phenotypes, could be used to identified the involve genes.

051. Obtaining F1 hybrids from habanero pepper (*Capsicum chinense*, Jacq.) for different purposes

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The hybridization in the traditional genetic improvement allows the obtaining of F_1 Lines of high yield and fruit quality, by taking advantage of the combinatorial ability and heterosis during the crossing between elite parents, particularly in autogamous species. For this, parents of different genetic constitution are crossed in order to achieve the transfer of desirable characteristics (genes) between the parents. In this species heterosis has been reported for yield ranging from 28% to 47%. In this study, 11 genotypes of the Habanero pepper germplasm bank conserved in laboratory 9 of the UBBM of the CICY (Center for Scientific Research of Yucatan) were used. Through the hybridization improvement method, 22 F1 Lines were obtained in the spring-summer period of 2019. The highest number of seeds for fruits was obtained when the RHC-05 and NKA-11 genotypes were used as progenitors, obtaining up to 52 seeds for fruits. The genotypes RCR-01, RNJ-04 and RES-08, for showing greater delay in flowering (late) require an outdated management in their establishment, with respect to the rest of the genotypes. As a result, it was also observed that the time in which the crosses are made should be when the temperature is cooler (25-30°C). Of a total of 1309 crosses, 216 crosses were achieved for a 16.5% effectiveness.
Keywords: Habanero Chile, hybridization, yield

052. Genetic transformation of sorghum by *Agrobacterium tumefaciens* with the mannese gene peroxidase *MnP2*

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Genetic engineering of plants is directed to genotype production which express characteristics of interest, through the plant genome integration of exogenous DNA. The search of alternatives for cell wall polymers degradation, in particular, lignin, seeks to reduce the costs production of the enzymes involved in their degradation, specifically, MnPs (Manganese peroxidases), which could become a versatile biocatalyst in the biotechnological processes (Okazaki et al., 2001; Van Aken et al., 2000). In the present work, we established as the main objective, the genetic transformation of sorghum via *Agrobacterium tumefasciens* with a gene which encodes for MnP2 from *Fomitiporia mediterranea*. In the first instance, we made an *in silico* analysis with Matras program to compare two MnP2 from *F. mediterranea* and *Phanerochaete chrysosporium*; the result showed that the two structures correspond to the same protein superfamily; although they share only 50.4% similarity in the amino acid sequence, the similarity of the secondary structure is 96.5% with a DMRS of 0.69 Å, which indicates, at least *in silico*, that the protein produced by the *FmmnP2* gene synthesized could be functional. After that, we designed pTM6 binary vector which contains *Fmmnp2* gen, encodes for Manganese peroxidases, under the control of CAMV35S promoter. For plant transformation, immature sorghum embryos were used, they were placed in the culture medium (MS with vitamins, pyridoxine, nicotinic acid, sulfad, BA, sucrose 30 g / L, pH 5.8) for three days in the dark. The infected sorghum explants were transferred to selection medium (MS supplemented with vitamins, pyridoxine, nicotinic acid, BA, sucrose 30g / L, pH 5.8), supplemented with 25 mg / ml hygromycin and 250 mg / ml cefotaxime 3-4 weeks until the appearance of calluses. With the results obtained in this work, we are achieving a new generation of transgenic plants capable of reducing lignin contents and therefore, improve their agronomic quality.

053. Determination of the function of the three genes of the bixin biosynthesis pathway in *Solanum lycopersicum*

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Bixa orellana (family Bixaceae) is a perennial tree species of great economic importance due to the high content of bixin, a red-orange pigment present in the aryl or in the cover of the seeds. This natural red-orange dye is used as a pigment compound in the food, drug and cosmetic industry and today its international commercialization has increased significantly. Bixin is an apocarotenoid that is derived from the oxidative cleavage of lycopene. The bixin biosynthesis pathway has been described for more than a decade. Jako et al. (2002) proposed the bixin biosynthesis pathway from the generation of an EST (Expressed Sequenced Tags) library, considering lycopene as a possible precursor. Subsequently, Bouvier et al. (2003) demonstrate the synthesis of bixin using three presumably Bixa orellana genes, different from those found by Jako et al. (2002), and genetically modified E.coli to produce lycopene as a heterologous expression system. So far, lycopene has been proposed as a possible precursor, as well as the participation of genes such as lycopene dioxygenase (*BoLCD*), bixin aldehyde dehydrogenase (*BoALDH*) and norbixin methyltransferase (*BoMTH*). However, other research groups have not found the same results regarding the involvement of these genes in bixin biosynthesis. The completion of the B. orellana transcriptome showed genes possibly involved in the bixin biosynthesis pathway, 5 BoCCDs (carotenoid cleavage dioxygenases), 2 BoALDHs (aldehyde dehydrogenases) and 3 BoMTHs (SABATH family Methyltransferases). These genes were abundantly expressed in seed and during bixin biosynthesis). Thus, the objective of the present work is to characterize the function of at least two combination of the three *BoCCD*, *BoALDH* and *BoMTH* genes in a heterologous organism, such as tomato (*S. lycopersicum*), a plant producing lycopene and other carotenoids. This will allow to confirm that the precursor of bixin is lycopene and will also clarify the participation of these genes in the biosynthesis of bixin.

054. Knockout of FAD2-1 gene in *J. curcas* using CRISPR/Cas9 to interrogate lipid biosynthesis pathway

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J. curcas is a plant distinguished by the high content of fatty acids harbored in its seeds and have potential in the production of biodiesel. However, high production of unsaturated fatty acids generates a disadvantage for biodiesel production wherein saturated, and monounsaturated fatty acids are preferred. The aim of this work is the characterization of callus and suspension cells and the construction of a vector designed to mutate the JcFAD2-1 gene that codes for the enzyme FAD2-1 by CRISPR-Cas9 system for upcoming genetic transformation essays. The explants used for the callus characterization were taken from cotyledonary leaves, then the callus is used for establishment of cellular suspensions. From tissue culture experiments both fresh and dry weight were determined, as well as the growth indexes of callus were calculated. Also, viable cellular suspensions were documented, with an inoculum of 0.25 g the growth phases were identified. For CRISPR guide RNA (gRNA) design, we used an online bioinformatic tool (CRISPOR) to select and evaluate in silico appropriated guides. gRNA includes AtU6 promoter, tracRNA and transcription termination sequence; the synthesis is based on the generation of inserts with homologous ends to the vector and cloning is made by Sequence and Ligation Independent Cloning (SLIC). PCR products accurate sizes for gRNA were detected by electrophoresis and further will be confirmed by sequencing analysis. In conclusion, these methods set the base for J. curcas genetic transformation from callus and cellular suspensions. Nevertheless, SLIC parameters must be standardize to ensure the correct assembly of the inserts within the vector employed.

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Keyword: *Jatropha curcas*; Establishment of cells suspension; Establishment of callus; CRISPR-Cas9; Fatty-acid biosynthesis.

055. Evaluation of molecular markers for resistance to *Fusarium oxysporum* in commercial varieties of *Solanum lycopersicum* L. as a tool for genetic improvement

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Tomato (*Solanum lycopersicum* L.) is an economically important crop worldwide and is one of the main export vegetables grown in Mexico. However, tomato is affected by the fungus *Fusarium oxysporum*, which is typically controlled using chemical compounds promoting the emergence of resistant strains. One of the strategies to cope against this pathogen is the use of resistant tomato varieties, which can be developed by detection of molecular markers. The aim of this work was to evaluate the dominant At2-F3/At2-R3 and codominant I-2 molecular markers linked to FOL1 (resistant to *Fusarium* race 1) y FOL2 (resistant to *Fusarium* race 1 and 2) genes, respectively, in commercial tomato varieties by endpoint PCR using previously reported primers. For that, we analyzed eight commercial tomato hybrids SVTE8444, SV8579TE, Valerio, Vanessa, Alvaro, SV3543TE, DRD-600, DRD-8551; and two differential cultivars, Bonny Best (susceptible to race 1, 2 and 3) and Walter (FOL2). All commercial varieties, but no Bonny Best and Walter, exhibited a 130 bp specific-band indicating the presence of At2-F3/At2-R3 marker linked to FOL1. For I-2 marker, SVTE8444, SV8579TE, Vanessa, Alvaro, DRD-600 and DRD-8551 showed two bands of 633 and 693 bp corresponding to the susceptible and resistance alleles, respectively, indicating they are heterozygous for FOL2. Valerio and SV3543TE exhibited susceptible and resistance FOL2 bands, in addition to one unspecific intermediate band. Moreover, Walter showed the resistance and unspecific bands, while no amplification was found for Bonny Best. At2-F3/At2-R3 and I-2 were amplified using at least 0.78 ng of genomic DNA per 20 µl PCR reaction, and showed a dynamic range for PCR amplification from 0.78 to 100 ng, with well-defined bands using 12.5 ng of gDNA. These results clearly demonstrate the feasibility of detection of molecular markers by endpoint PCR as a useful tool for screening of tomato genotypes that can be implemented in genetic improvement programs.

056. Gene editing of pbs1 gene from soybean (*Glycine max*) for the control of fungal infections

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Asian soybean rust, whose causal agent is *Phakopsora pachyrizi*, generates important economic losses worldwide1. A recent approach suggests a novel control strategy. Indeed, in *Arabidopsis* the disease resistance protein RPS5 is activated by PBS1, which is in turn activated by the *Pseudomonas syringae* AVrPphB protease. Recently, the recognition range of this protein has been broadened via decoy engineering thus recognizing other bacterial effector proteases and even viral proteins2. The objective of this work was editing the homologous pbs1 gene in order to enable its activation by fungal effectors, which could lead to increased tolerance to such fungi. First, the PBS1 homolog was searched for in the soybean genome. The corresponding gRNA was designed using the CRISPR-P 2.0 software; this sequence, together with the Cas9 gene, was introduced in the CRISPR-CAS9 expression vector for edition. In addition, we analyzed the proteasome of *P. pachyrhizi* in UNIPROT, CLUSTAL-W and MEROPS databases, to identify proteases that could potentially activate soybean PBS1. Additionally, the canonical target sequence for different aspartyl proteases was introduced as DONOR, which could in principle broaden the recognition range of PBS1. We have found that 12 amino acid residues correspond to the canonical target sequence of aspartyl protease from *P. pachyrhizi*. We have also analyzed the soybean genome and identified 3 GmPBS1 homologs to A. thaliana PBS1 in chromosomes 8,10 and 20. This DONOR sequence was synthesized by molecular assembly and cloned into the vector PCR8GWTOPO. These vectors were used to transform 140 soybean embryos via biolistics. The Cas9 gene was detected via PCR in DNA from transformed soybean plants. Finally, the soybean transformation efficiency was 30/140, which is high relative to other methods. Currently, we are analyzing the molecular profile of the candidate edited plants.

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057. Global gene expression and prediction of metabolic pathways involved in defense responses of *Persea americana* Mill to Fusarium dieback.

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Transport and trade of diverse products in the world have contributed to dispersion of a wide organism variety to new areas. Polyphagous trunk and branch borer beetles like Euwallaceae sp. nr. (PSHB) and Euwallaceae sp. nr fornicatus (KSHB) are an example. These are invasive pest origin from Asian with high expansion capacity that are associated symbiotically with some fungi, including two from genus Fusarium and that cause a necrotic disease known as Fusarium Dieback (FD). This disease affects more than 300 plant species hosts; among them avocado (Persea americana Mill., Lauraceae), which is especially susceptible to KSHB/Fusarium kuroshium complex. Its expansion in California (the most important avocado producing state in the US) has increased rapidly since 2014. Moreover, since it has a wide hosts variety, it could eventually expand in Mexico and affect both forest areas and producing areas of avocado. In this study, the caused effect of this disease is analyzed by gene expression and the chemical composition involved in plant defense and metabolites biosynthesis, from transcriptomic data (getting by RNA-Seq) and metabolomic data (obtained by organic extracts) from healthy avocado trees and trees infected by Fusarium kuroshium from an orchard in California.

058. Early transcriptomic changes in *Solanum lycopersicum* and *Solanum arcanum* induced by the pathogen *Clavibacter michiganensis* subsp. *michiganensis*

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Bacterial canker of tomato, is one of the most important bacterial diseases of this crop, which produces important economic losses worldwide. The canker is caused by the actinomycete *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). Unfortunately, there are no effective control strategies and only few preventive methods work efficiently. Currently, several wild tomato species such as *Solanum arcanum* and *Solanum habrochaites,* have been described with different resistance levels to *Cmm*, being *S. arcanum* LA2157 the most resistant species. However, these are not edible species. The study of these tomato wild species represents an important approach for identifying a natural resistance source that can be used in commercial tomato crops for controlling bacterial canker. Therefore, the aim of this work is to identify tolerance-related genes to *Cmm* by comparing the transcriptomic profile obtained with RNA-Seq of *S. arcanum* LA2157 (resistant) and *S. lycopersicum* (susceptible) during their first 24 hours of interaction with the *Cmm* pathogen. We accomplished three different strategies for the bioinformatic analysis: mapping to the reference genome SL3.0, Semi *de novo* transcriptome assembly and *de novo* transcriptome assembly. Our preliminary results suggest that both tomato species exhibit a high amount of differentially expressed genes from 0 hours to 24 h after the *Cmm* challenge. The highest differences were showed at 8 h after challenge. Particularly in the wild species *S. arcanum* LA2157, we found several enriched GO terms that are related to defense response; meanwhile, in the commercial tomato species these groups were not equally enriched. The future work relies on the functional validation of the putative tolerance-related genes to *Cmm* by virus-induced gene silencing approach in wild tomato species and finally to overexpress the candidate genes by gene editing (CRISPR/Cas9).

059. Structure, phylogeny and expression analysis of genes encoding PAMP recognition receptors in banana

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Banana stands out as a food security crop. Around four hundred million people in developing countries depends on the production and commercialization of banana. Currently, the banana production relies on the intensive and extensive applications of fungicides to control *Pseudocercospora fijiensis*, a fungus that causes the black Sigatoka disease. The chemical control of this disease is highly expensive (US$1000/ha) and with negative consequences to the environment and human health. Recently, the banana genome of a wild genotype with resistance to black sigatoka was sequenced. The availability of this sequence should facilitate the study of genes involved in plant immunity. PAMP (pathogen associated molecular patterns)-triggered immunity is an effective defense response mediated by Pattern recognition receptors (PRR) in plants. Some of these PRR proteins confer broad-spectrum resistance. Therefore, the characterization of this type of receptors should provide valuable insights for the genetic improvement of crops.

In this study, we are characterizing the structure, phylogeny and expression of PRR genes with LRR domains. So far, we have retrieved from the banana genome the sequences of several members of this family and performed a comprehensive structural and phylogenetic analysis. Our bioinformatic analysis suggest that we have found a group of LRR homologs that are similar to LRR genes involved in disease resistance.

060. Transcriptomic characterization of the *Arabidopsis thaliana eca2* mutant reveals modification of cuticular- and defense-related genes.

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The cuticle that covers the surfaces of the plant aerial organs, is composed of cutin and waxes. It plays diverse functions such as maintain organ integrity, prevention of uncontrolled water loss and act as a barrier to protect against pathogen invasion. However, the molecular mechanisms underlying the cuticle monomers perception that regulates plant immunity remain unclear. The mutant *eca2,* that constitutively express the MAMP-induced early response gene *ATL2,* shows an increase permeability of the cuticle as well as a reduction of both cuticular components waxes and cutin. Additionally, *eca2* showed resistance to a fungal pathogen *Botrytis cinerea*, a bacterial pathogen *Pseudomonas syringae* and an herbivorous insect *Spodoptera littoralis*. To try to understand these phenotypes, we used RNA-seq to elucidate gene expression changes in *eca2*. Were identified 1,184 DEGs, including 817 upregulated and 367 downregulated genes in *eca2* compared to the WT plants. Several cutin and wax biosynthesis and transport of cuticle genes were identified as DEGs such as *BDG*, *LACS2*, *LCR*, *HTH*, *LACS1, LACS2, WAX2, CER3*, and *CER1.* Other genes related to cell wall as expansins were part of the upregulated genes as well. Additionally, several well-characterized hormone-responsive genes, including JA response, ET responsive, ABA responsive, and SA responsive were identified. KEGG pathway enrichment analysis showed that lipid and carbohydrate metabolism, as well as signal transduction and immune system was significantly enriched. These results may help us to reveal the mechanisms underlying the cuticle permeability, its biosynthesis and the initial events of plant defense against these pathogens that take place in the cuticle surface in *eca2*.
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061. Resistance to *Candidatus* Liberibacter solanacearum and Genetic Diversity Analysis in tomato (*Solanum lycopersicum* L.)

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Candidatus Liberibacter solanacearum (*C*Ls) is a plant pathogen associated with serious diseases affecting potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) crops. Insecticides are applied to reduce the presence of the insect vector, *Bactericera cockerelli* (*Bc*), due to the absence of commercial cultivars resistant to *C*Ls. Seven tomato genotypes underwent in a resistance to *C*Ls assay and genetic diversity analysis: Río Grande, Moctezuma, Marmande, DRK 2180, La Roca, Bonny Best and UAS 2016. Resistance assay was performed in Culiacan, Sinaloa, Mexico using a randomized blocks design with four replications. *C*Ls bacteria was inoculated by *Bc*. Assesment of genetic diversity was determined selecting 15 SSR markers and a cluster analysis was done by unweighted pair-group method with arithmetic averages (UPGMA). UAS 2016 and Marmande showed significantly a better resistance response to the presence of *C*Ls compared to Rio Grande and Moctezuma. DRK 2180, La Roca and Bonny Best didn't show a better response compared to Moctezuma but they were significantly better compared to Rio Grande. Total genetic diversity value was 0.58 and genetic diversity among subpopulation was 0.30. The resulting dendogram clustered genotypes according to fruit shape. UAS 2016 and Marmande has been identified as candidates for plant breeding programs.

062. Functional analysis of the *PvDIR1* gene in transgenic roots of *Phaseolus vulgaris* under nodulation conditions

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In legumes, gram-negative soil bacteria called rhizobia induce the formation of nodules, a specialized organ in which atmospheric nitrogen is fixed by the bacterial symbiont. The establishment of this interaction requires an exchange of chemical signals between roots of the plant and the microsymbiont. Previously in our research group, a transcriptomic analysis by RNA-seq of transgenic *Phaseolus vulgaris* roots with loss-of-function of *PvRbohB* gene in symbiosis with the rhizobacteria *Rhizobium tropici* or with the arbuscular mycorrhizal fungi *Rhizophagus irregularis* was performed. Several differential expressed genes were identified in the initial stages of nodulation and mycorrhization in *P. vulgaris*. Within this gene battery, the *PvDIR1* gene was upregulated in nodulated and mycorrhized control roots and its expression was affected by the *PvRbohB* silencing. *DIR1* (DEFECTIVE IN INDUCED RESISTANCE1) is a non-specific lipid transfer protein that is required for the generation and/or translocation of mobile systemic acquired resistance signals in *Arabidopsis thaliana*. However, the participation of *DIR1* in mutualistic plant-microbe interactions has been poorly explored.
Functional analysis by reverse genetics showed that the transcript level of *PvDIR1* in *PvDIR1*-RNAi transgenic roots of *P. vulgaris* was significantly lower both in uninoculated and inoculated roots with *R. tropici*. The rhizobial inoculation induced an increase of *PvDIR1* transcript level at 7 days post-inoculation (dpi) in roots silenced, unliked that control roots decreased its transcript level. The silenced roots showed a root weight reduction of approximately 53% and 72% with respect to inoculated and uninoculated control roots at 7 dpi. In addition, gene silencing of *PvDIR1* decreased 53% in the number of nodules compared to the control. These results suggest that *PvDIR1* could play a role in the early stages of nodulation in *P. vulgaris*, as well as in the growth and development of the roots.

063. Identification of chayote (*Sechium edule*, Jacq. Sw.) genes differentially expressed during the infection with *Phytophthora capsici* L.

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Chayote is a Cucurbitaceae of great importance in Veracruz, at the time of increased rainfall it is affected by *Phytophthora capsici*, which causes root rot, wilting and plant death. Its pathogenicity has been evaluated under different conditions; however, there is no information about the genes that are expressed in the plant in response to the attack by this oomycete. The aim of this study was to identify chayote genes related to the establishment of defense mechanisms in plants inoculated with *P. capsici*. Fifteen plants of 1 mo were inoculated, placing a disc of 0.5 cm in diameter of mycelium of *P. capsici*, to the mock inoculated plants only V8 agar was placed. The plant material was collected and frozen immediately with liquid nitrogen, at 0, 12, 24, 48 and 120 hours post-inoculation (hpi), a part of the stem was obtained at the inoculation site and 20 cm apart, in the direction of apical shoot. Total RNA was extracted, cDNA was synthesized and a series of PCRs were performed with primers designed from conserved regions of 12 genes involved in plant defense (two MAPKs, three ACSs, one ETR1, one CTR1, three NBS-LRRs, one WRKY and one Chitinase). The obtained amplicons were visualized in 2% agarose gels, purified and sent to sequence. The sequences obtained were characterized with the BLAST tool of the NCBI. The identity of the 12 genes was corroborated. ACS6 showed greater expression in the local tissue inoculated with the oomycete, compared to the mock inoculated at 48 hpi and at 120 hpi showed an opposite expression in the systemic tissue. ETR1 and Chitinase were expressed more in the local mock inoculated tissue than in the inoculated plant, at 48 and 120 hpi. These genes participate in the ethylene signaling pathway, which may be involved in the development of chayote wilt.

064. Analysis of the MADS-BOX (AGL) family of transcription factors in roots and nodules of common bean and other legumes

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The MADS-box (AGL, AGAMOUS-Like) family of transcription factors (TFs) has been defined based on primary sequence similarity amongst numerous proteins from a diverse range of eukaryotic organisms including yeasts, plants, and mammals. The plants' MADS-box proteins form numerous high-order protein complexes, with different DNA-binding specificities that play a pivotal role in the regulation of target genes and plant morphogenesis. Plant AGL TF genes have been extensively characterized mainly as regulators of reproductive development, flower transition, and organ identity; however, many of these TFs are also expressed and have regulatory roles in other tissues. Recently the role of Arabidopsis AGL genes in root development has been reviewed (Álvarez-Buylla et al. 2019). Our analysis in common bean (*Phaseolus vulgaris*) revealed 17 (from 93) AGL genes are highly expressed in roots and nodules elicited in the rhizobia symbiosis (Íñiguez et al. 2015). In this work we performed a phylogenetic analysis of AGL genes from Arabidopsis and the legumes: common bean, soybean, Medicago and Lotus. Certain clades from the phylogenetic tree cluster AGL genes expressed in roots from the different species. Expression analysis (qRT_PCR) confirmed the high expression of seven *P. vulgaris* AGL genes in root/nodule during different stage of the rhizobia symbiosis. To investigate the role of common bean AGL as regulators of the symbiotic N_2-fixation (SNF) process we used the reverse genetic approach to generate "composite" plants -transgenic roots/nodules- with overexpression or silencing of a selected AGL gene. Phenotypic analysis of the composite plants in symbiosis with rhizobia would allow proposing their regulatory role and relevance for SNF.

065. *Bacillus velezensis* 83 promotes plant growth of *Arabidopsis thaliana* L. by a mechanism dependent of the cytokinin signalling pathway

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Bacterial isolates from diverse natural environments can be used as biofertilizers or biopesticides, because they are able to increase the plant growth. Well directly, by modifying plant signaling and/or metabolism, also indirectly, counteracting the growth of plant pathogens. *Bacillus velezensis* 83 (*Bv*-83) is a bacterial strain isolated from la phyllosphera of mango, that is the bio-active component of a biotechnological biofungicide marketed as *Fungifree AB*®, whose use favors the healthy growth of different crops. In this work, we describe a direct mechanism of plant growth promotion by *Bv*-83 on *Arabidopsis thaliana* L. (*A. thaliana*). The results showed that *Bv*-83 promotes the accumulation of plant biomass altering seedling morphology, through the colonization of plant tissues, also by the production of volatile and diffusible compounds. An analysis of expression with plant reporting lines revealed that direct contact with the bacteria causes a greater mitotic activity (*CycB1;1:uidA*), and a greater activity of cytokinin signaling pathway (*ARR5:uidA*), in both shoot and root meristems. However, *Bv*-83 was unable to promote plant growth of double mutants affected in pairs of cytokinin receptors (*cre1-12 ahk2-2*, *cre1-12 ahk3* and *ahk-2 ahk3*), indicating that cytokinin are involved in the control of the effects on plant growth of these bacteria. Finally, GUS activity assays proved that *Bv*-83 infection induce the expression of two genes related to phosphate transport in the root tissues (*AtPT1:uidA* and *AtPT2:uidA*). All together, our results support that *Bv*-83 promotes plant growth by increasing cell proliferation dependent of *CYCB1* expression, which requires the functioning of cytokinin receptors encoded by *AHK2*, *AHK3* and *CRE1*/*AHK4* genes which cause a greater expression of *ARR5* in shoot and root meristems and an increased expression in the root of the phosphate transporters *ATPT1* and *ATPT2*.

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066. Study of the interaction between *Fusarium falciforme* and *Arabidopsis thaliana*

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Background: *Fusarium falciforme* is a species belonging to the *Fusarium solani* complex (FSSC) that has been reported as an opportunistic pathogen of humans and animals, and has recently been considered a phytopathogen. Due to the lack of insulation, there is still scarce information about the specific mechanism used by *F. falciforme* to cause damage to the plant, so using a widely studied model such as *Arabidopsis thaliana*, the machinery of infection exerted on its hosts will be established under different pH conditions.
Objectives: evaluate the interaction between *Fusarium falciforme* and *Arabidopsis thaliana* under different pH conditions
Methods: Interaction bioassays were carried out between *F. falciforme* and *A. thaliana* Col 0 and its transgenic lines *DR5::uidA* (auxin response reporter line), *PRZ1::uidA* (meristem identity reporter line) and *CYCB1::uidA* (cell cycle reporter line) under diverse pH conditions.
Results: At acid pH (pH 5), *F. falciforme* has a phytopathogenic effect on *A. thaliana,* affecting root elongation, decreasing auxin production (effect observed in the transgenic line DR5::ui) and affecting cell division (effect observed in the transgenic lines *PRPZ1::uidA* and *CYCB1::uidA*). At basic pH (pH 11), *F. falciforme* exerts a symbiotic effect since an increase in the normal growth of the *A. thaliana* seedlings is observed compared to the control at pH 7. *F. falciforme* is able to exert an antagonistic interaction and/or symbiotic that depends on environmental factors such as pH.

067. Characterization and evolution analysis of NBS-LRR gene type in the transcriptome of *Agave tequilana*

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Agave tequilana is a widely cultivated to produce tequila. The plantations of these plants are vulnerable to pathogens and adverse conditions. One of the main response mechanisms against of the pathogen infection involves to the NBS-LRR genes. Different strategies, mainly by use the NBS domain, have been published for the isolation of these genes. In addition to genomic analysis, transcriptomic databases have also been used for the isolation of NBS-containing sequences. In this study, NBS-LRR type sequences were identified in the Transcriptome Shotgun Assembly (TSA) of *A. tequilana* based on a partial region in the NBS domain conserved using the TSA BLAST search. A total of 46 TSA sequences was retrieved and clasified within of the no-TIR class and subclassified into five subclasses (CNL, CN, NL, N and L). The identified sequences were characterized based on physicochemical properties, gene structure and motif analysis, functional annotation and gene ontology. In general, all sequences analyzed encode functional NBS-LRR proteins. Phylogenetic analysis showed that these genes were clustered into five groups (group I-V). The gene structure and motif composition were highly conserved within of each group, but divergent among then. These groups are under a diversification pressure (K_a/K_s rates<1) with exception of the group V (K_a/K_s rate = 1.23) which is of recent formation (9 Mya). The expression response during a pathogenic infection was experimentally verified for the three mayor groups. An *Agave* pathogenic *Lasiodiplodia* strain was used to induce a stress response on *A. tequilana* leaves. The highest NBS-LRR gene transcript induction was obtained at 48 h post infection for the group I and V, the group II have a eartly induction at 6 h. Expression profile was different among the different NBS-LRR gene families suggesting that each group may have a specific function in the defense response to pathogens.

068. Identification of pathogenic genes *a*ssociated with vanilla root and stem rot in *Fusarium* fungi isolated from *Vanilla planifolia.*

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Vanilla planifolia is one of the most important orchids, its extract is sold as the second most valuable vegetal product worldwide and its biological origen is Mexico. The main biological challenge with its production is the root and stem rot disease, caused by fungi of genus *Fusarium*, most commonly by *Fusarium oxysporum,* a fungus that develops close host-pathogen interactions, holding high host-specific genetic variability. Transposable elements play an important role in this ongoing arms race, specially a miniature inverted-repeat transposable elements family (mites), related with genes capable of confer pathogenicity by horizontal gene transfer in different strains. Our goal in this work is to identify specific *Fusarium* pathogenic genes (homologous and novel). We isolated 76 morphologically different axenic fungal endophytes from healthy and infected vanillas, from this, we identified 9 different genera with common molecular markers. Five *Fusarium* strains were selected for whole genome sequencing. A good-quality assembly of fungi isolated from healthy and symptomatic vanilla has been generated for the first time. Candidate pathogenicity genes were identified by comparative genomics, functional annotation of the assemblies and prediction of effector and secreted proteins. This work seeks to develop a quicker and easier pathogen identification in vanilla plantations and eventually the reduction of the disease on the field.

069. Edited strains in an APSES Transcription Factor of ambrosia Fusarium present different level of virulence against *Populus nigra.*

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Ambrosia Fusarium forms symbiotic relationship with invasive ambrosia beetles such as those belonging to the *Euwallacea fornicatus* species complex which attack declining or dead trees, but some attack healthy trees in native and invaded areas. The beetle inoculates *Fusarium sp.* for nutritional purpose into its hosts, where the adult and larva beetles feed on the fungal mycelium. Simultaneously, the fungus provokes Fusarium dieback disease by interrupting the nutrients and water transport in branches of infected trees causing canopy loss, and in some cases tree mortality. The ambrosia complex has a wide range of plant host including native, agriculturally important and common street trees. Among these hosts are species of *Populus*, *Salix*, *Persea*, *Ricinus*, *Quercus*, *Liquidambar*, *Macadamia* and others. Despite the relevance of the ambrosia Fusarium species, there is not information about the molecular mechanism of their pathogenesis and defense response that the pathogen triggers in the plant, which can be valuable for the implementation of strategies for the disease control.
In this sense, by means of a transcriptomic analysis, we selected an APSES transcription factor in order to generate edited mutants in ambrosia Fusarium by the CRISPR/Cas9 system to evaluate their possible role in virulence and development. An ambrosia *Fusarium solani* strain isolated from *Xylosandrus morigerus* ambrosia beetle is used as a model. We obtained four edited strains, in which, the sequence analyses revealed deletions, mainly, in the promoter region. Infection assays using the pathosystem *Populus nigra-F. solani* indicate that two edited strains are less virulent and two strains are more virulent than WT. Thus, by genome edition we can generate fungal strains with different level of virulence. These strains will allow us the identification of defense pathways triggered by ambrosia Fusarium in different hosts and the transcriptional profiles in host and pathogen during the infection process.

070. Participation of TOR kinase in the interaction *Arabidopsis thaliana*-*Azospirillum brasilense* Sp 245

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The target of rapamycin (TOR) kinase is a master regulator of metabolism, translation, and transcription to fuel celular proliferation and organismal development and growth. TOR is present throughout the eukaryotes, that kinase is a member of the phosphatidylinositol 3-kinase (PI3K)-related kinase. The TOR functions were first described in yeasts and mammals, in which TOR is found in two protein complexes: the rapamycine-sensitive TORC1 and the rapamycin-insensitive TORC2. The first contains the TOR kinase and the core components, regulatory-associated protein at TOR (RAPTOR) and lethal with SEC13 protein 8 (LST8), while TORC2 is composed of rapamycin-insensitive companion of mTOR (RICTOR), TOR and LST8. Homologous proteins of complex TORC1 have been found in plants, but to date there is not evidence of RICTOR existence. Plant TOR is activated by nutrients, light and phytohormones such as auxins. A few TOR substrates have now been identified in plants, including ribosomal protein S6 kinase (S6K), the type 2A phosphatase associated protein 46 kDa (TAP46), the PYL ABA receptors and the transcription factor E2Fa. On the other hand, *Azospirillum brasilense*, is a plant growth promoting rhizobacteria (PGPR) that has been widely used in crops, because it increases grain yield. The model plant *Arabidopsis thaliana* was chosen as host plant to gain an insight into the molecular mechanisms that govern this interaction. *Arabidopsis* seedlings inoculated with *A. brasilense* showed alterations in radicular architecture, phenotype has been mainly attributed to bacterial auxins. Recently, was reported that *Arabidopsis* primary root growth is regulated by TOR, at level of proliferation and elongation cellular. In the present study we are hypothesized that TOR is required for that *A. brasilense* stimulates lateral roots development in *Arabidopsis*, for which we used the second generation TOR inhibitors: AZD-8055 and Torin1, in addition of the conditional *tor-es1* mutant of *Arabidopsis.*

071. *In vitro* effect of *Fusarium verticillioides* fungalysin on the modification of maize (*Zea mays L*.) and *B. cereus B*25 chitinases

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Zea mays L. is one of the most important cereals in the world, being Sinaloa the main producing state. Maize is susceptible to various diseases caused by phytopathogenic fungi, one of them is *Fusarium verticillioides* (*Fv*), which causes stem and root rot. *Bacillus cereus B*25 has shown antagonistic effect against *Fv* through the production of antifungal compounds such as siderophores, antibiotics and chitinases, these enzymes hydrolyze the chitin in the pathogenic fungi cell wall.

*B*25 chitinases (ChiA and ChiB) are induced in the presence of the fungus and fungal lysates. Likewise, maize is capable to produce chitinases (ChitA and ChitB) and induce plant defense when invaded by fungal pathogens. However, *Fv* produces chitinase modifying proteins (*Fv*-cmp) or fungalysins, which separate the chitin-binding domain and the catalytic domain of the plant chitinases causing to lose their ability to bind chitin and hydrolyze it.

In our proposed model of tripartite interaction (maize-*B*25-*Fv*), *B*25 chitinases may not be modified by fungalysin because they don't have the protein domain that fungalysin recognizes. It has been postulated that the bacterial chitinases may degrade chitin in the fungal cell wall and elicit plant defense response.

This work aims to demonstrate *in vitro* that fungalysin does modify plant chitinases but not bacterial chitinases. For the *in vitro* modification, a fungal protein extract was obtained from hybrid white corn seeds of Asgrow Garañón inoculated with *Fv* conidia. The *Fv*-cmp assay test was carried out by assaying separately plant and bacterial chitinases with the fungal protein extract. The protein modification was visualized by SDS-PAGE and Western-blot. According to our results, the ChitA and ChitB plant chitinases were modified in the presence of the fungal protein extract, and bacterial chitinases were not.

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072. Genome wide identification and analysis of Sucrose non-fermenting-1 (SNF1)-related protein kinases (SnRK) in bean and their responses during mycorrhizal and rhizobial symbiosis

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Sucrose non-fermenting-1 (SNF1)-related protein kinases (SnRKs) which, as the name implies, are a homologous group of fungic SNF1 responsible for carbon regulation. A total of 38 genes that make up this family of kinases have been described and characterized in arabidopsis, which are divided into 3 subfamilies: SNRK1, SNRK2 and SNRK3. The SNRKs are responsible for metabolic and stress signaling, this makes them potential candidates to be used in the manipulation of plants to improve performance. Homologous genes have been found and characterized in plants such as rice and in some legumes such as soybeans, but up until now there has not been an in-depth analysis of this family in legumes such as common bean (*Phaseolus vulgaris*). Therefore, an in-depth analysis of the SNRKs in common beans revealed presence of a total of 42 genes which are classified into three subfamilies. The domain analysis showed the conservation of domains such as SnRK1 – KA1 domain, SnRK2-OST domain and SnRK3- NAF/FISL domains as characteristic of each of the sumfamilies. Phylogenetic tree showed grouping of the genes into 4 clusters, motif and exon-intron analysis further revealed the differences and similarities among the family of genes. Global transcriptomic analysis of *P. vulgais* under rhizobial and mycorrhizal symbiotic conditions showed differential expression of SnRK1, 22 SnRK3 family members as compared to 6 SnRK2 family. These studies could serve as the fundamental data for further characterization of SnRK family genes under symbiotic conditions.
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073. Down-regulation of a *Phaseolus vulgaris* aquaporin Pvpip2-4 affects the nodulation process

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Plant aquaporins are a large family of proteins solutes transporters (water, sugar and NH4) that play an important role in several physiological processes in living organisms. On the other hand, hydrogen peroxide (H_2O_2) levels and transport have been related with plant growth, development, biotic, and abiotic stress responses. It has been proposed that aquaporins can also transport H_2O_2, regulating the subcellular distribution and thus signal strength. However, little is known about this process. It´s well known that reactive oxygen species (ROS) are highly involved in polar growth, but also during the mutualistic interactions such as the rhizobia-legume or mycorrhizal association. ROS generated in the apoplast by NADPH oxidases and SOD activity, such as H_2O_2, need to be transported from the extracellular side to the cytoplasm. However, we know little about this process. Thee functional role of aquaporins in *P. vulgaris* and their potential role to transport H_2O_2 in root hairs during the polar growth and rhizobia-legume interaction, has been poorly studied. In this study we determined the role of *PvPIP2-4*, a gene encoding for an aquaporin that could be involved in the H_2O_2 transport. By silencing and overexpression of the gene in *Phaseolus vulgaris*, we have also evaluated the effect on the nodulation process. We have found that *PvPIP2-4*, depict an early increased transcript accumulation in roots inoculated with *Rhizobium tropici* CIAT899; however, at later stages, the level of transcript decreased considerably. These results, and data describing the subcellular localization, nodulation and nitrogen fixation phenotype, will be presented and discussed.
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074. Expression study of the *ROPs* genes in the legume *Lotus japonicus*

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Nitrogen (N) is essential for plant growth and production because it is required in large quantities to synthesize several molecules indispensable to life, like DNA or proteins. However, nitrogen availability is limited in many soils throughout the world, which causes modern agriculture to overuse industrial fertilizers, omitting their strong negative impact on the environment. For this reason, it is crucial to identify alternative sources of fertilizers. The biological fixation of nitrogen could be an option. Plants like legumes have co-evolved with specific soil bacteria towards a symbiotic relationship, through which the host plant obtains ammonium from the bacteria in exchange of a carbon source and favorable environment to fix nitrogen. These bacteria enter the plant through root hairs, which is an active process requiring proper reorganization of the plant cell cytoskeleton. The goal of my project is to, during the entry of symbiotic bacteria in the root hair, evaluate and quantify the expression of the *ROPs* genes, codifying master regulators of the cytoskeleton rearrangement. This approach is being conducted in the legume model *Lotus japonicus*. Plants will be transformed with constructs containing the promoter of each *ROPs*, driving the expression of the *GUS* gene, allowing us to draw a spatio-temporal map of these genes expression in the root. At the same time, I will assess what is known about the expression of this family in other species like *Arabidopsis thaliana*, *Medicago truncatula* and *Glycin max*, using a data mining approach to exploit the transcriptomic data sets already available. My results will enable to identify which of the *ROPs* genes are involved in the early infection process initiating the symbiotic nitrogen fixation.

075. Endophytic *Lupinus* bacteria promoting growth of *Arabidopsis* in heavy metal stress.

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The bacteria living within plant tissues are called endophytic bacteria. Some endophytic bacteria have been classified as plant growth promoting bacteria (PGPB), furthermore, some of these bacteria have been reported as protective agents against abiotic stress and help to detoxify harmful compounds like Heavy Metals (HM). Our work group is interested on the study of endophytic bacteria from native plants that grow in soils rich in HM. In the present report, we describe endophytic bacteria, isolated from *Lupinus* plants, with the capacity of promoting growth of *Arabidopsis* in HM stress. Samples were obtained from mining tailings located at Morelos, Zacatecas. The endophytic bacteria were isolated from stems, leaf and roots tissues of *Lupinus*. The samples were superficially sterilized with 70% ethanol, 3% sodium hypochlorite and TWEEN 80 for 5,10 and 5 min, respectively and rinsed tree times with sterile water. Finally, tissues were crushed in NaCl 0.9%, and centrifuged 10000 rpm for 5 min, supernatant was recovered, diluted (10^{-1} - 10^{-4}) and inoculated by plate extension on TSA medium. We determined the Minimal Inhibitory Concentration for eight heavy metals of 15 endophytic bacteria isolated. It was observed that three microbial isolates have the highest HM resistance (140 mM AsV, 30 mM Aslll, 1 mM Co, 3 mM Cr, 6 mM Cu, 4 mM Zn, 2 mM Cd, 40 mM Pb and 2 mM Hg), and were selected to be identified. *Shingomonas* sp., *Shingopyxis alaskensis* and *Sphingomonas melonis* were identified using 16S ribosomal gene molecular phylogeny reconstruction. To evaluate plant growth promotion, we used an *in vitro* bacteria-plant system supplementing with 80 µM As, 20 µM Hg or 800 µM Pb. We found that all tree endophytic bacteria promotes *Arabidopsis* growth on all heavy metals evaluated. In conclusion, the endophytic bacteria isolated from native plant that grows in mining tailings have the capacity to promote growth under stress by As, Hg and Pb in a bacteria-Arabidopsis heterologous system.

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076. Metabolic changes during symbiotic nitrogen fixation in *Phaseolus vulgaris* nodules

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We focused on determine the role of folates and one-carbon (1C) metabolism *R. etli* and *P. vulgaris* symbiosis. Folates are highly hyperaccumulated in nodules, and we have collected evidence that folate biosynthesis is highly upregulated during this interaction. Not only folates are hyperaccumulated, all folate precursors occur in very high amounts. Pteridines and PABA also hyperaccumulated in nodules, pointing to both an upregulation of folate biosynthesis and a bottleneck in the synthesis. Pteridines found in nodules can be classified as biosynthetic (neopterin, monapterin, hydroxymethylpterin), and those product of pteridine and folate degradation (carboxypterin, pterin aldehyde and pterin); nodules accumulate 32 % of biosynthetic pterins, while 68 % are product of folate turnover. The expression and enzyme activity of the two committed steps of folate biosynthesis were also modulated in nodules. We have also profiled free amino acids, those related with 1C metabolism (Ser, Gly and Met) that are accumulated the highest in nodules of 21 days after infection; interestingly, non-fixating nodules ($NifA^-$) accumulated more Ser that nodules from fixating control interactions (CE3). Ser is one 1C donor for folates, which then serve as cofactor for many 1C transfer reactions, suggesting that differences in N fixation cause changes in 1C metabolism in nodules. Folate and 1C metabolism are highly compartmentalized; we have measured folates in mitochondria, plastids, and enriched cytosol. Surprisingly, the majority of the folate pool seems to be cytosolic. We discovered that folates were also transported within *P. vulgaris* xylem, for the first time we have detected, characterized, and quantified this vital cofactor species in the xylem. Previous proteomic studies have found folate-utilizing enzymes in xylem, all these results have implications about the presence of 1C metabolism in vascular contents and raise questions about its role in plants.

077. Endophytic *Beauveria bassiana* (Hypocreales: Cordycipitaceae) promotes early flowering and drought tolerance in *Zea mays* (Poales: Poaceae)

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B. bassiana is an entomopathogenic fungi that establishes endophytic symbiosis with plants for mutual benefits; as the plant gets protection against biotic and abiotic stresses and improves growth, the fungus gets protection against adverse conditions. Effect of endophytic colonization of *B. bassiana* strains on *Zea mays* growth, crop production and drought resistance were evaluated in this study. *Z. mays* seeds were inoculated with *B. bassiana* GHA, PTG4, and PTG6 strains with an initial concentration of $1x10^6$ blastospores/ml, using methylcellulose or corn starch formulations to protect conidial viability. Endophytic colonization was confirmed by isolating *B. bassiana* from plant tissues put onto PDA agar plates. Growth improvement was evaluated by recording plant height and flowering time, whereas drought tolerance was measured by stopping water supply for ten days, after which, water was provided, and vigor determined 24 h later. Guttation is a physiological mechanism that transport water and nutrients through the plant vascular system. Several signal transduction and transport proteins are found in guttation liquid therefore guttation liquid was used for protein analysis, inferring that its composition might be differentially regulated during plant-microbe interactions. Hundred percent of evaluated roots were colonized by fungus regardless of adherent or strain used, 73-74% using MC and CS respectively, from stems; and 66% with MC and 48% with CS from leaf tissues. *B. bassiana*-treated plants flowered earlier and showed tolerance against drought. Preliminary results in protein analysis showed proteins of 80 to 100 kDa. Taken together, results demonstrate that *B. bassiana* seed inoculation improves corn yield and drought tolerance.

078. The volatiles from *Trichoderma atroviride* improve growth of *Arabidopsis* seedlings through regulation of sucrose transport and metabolism.

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Different bacterial and fungal species produce a wide variety of volatile organic compounds (VOCs) that can be perceived by plants to modulate its metabolism and growth. Free-living microbes like filamentous fungi of the genus *Trichoderma* can produce plant growth-promoting compounds, which have the capacity to increase biomass production and alter root architecture. In addition, many *Trichoderma* species colonize and grow in association with plant roots. This implies a strong demand for carbon resources derived from photosynthesis, but the type of sugars transported among tissues, the transporters involved, and the possible exudation of these substances into the rhizosphere remains unknown.

Based on this information, the effects of volatiles from *T. atroviride* on *Arabidopsis* growth were tested in a closed co-cultivation system *in vitro* with varied sugar supplements. *Trichoderma* VOCs increased biomass production and the formation of lateral roots in medium with limited supply of sucrose, which correlated with a higher content of chlorophyll, the amount of sucrose and glucose available in both tissues and root exudation of sugars. The long-distance transport of assimilates from leaves to the root apparently occurs through SUC2 and it was possible to identify the 6-pentyl-2H-pyran-2-one (6-PP) as one of the volatiles responsible for the regulation of this sugar transporter. Moreover, *Trichoderma* volatiles modify the expression of sugar efflux transporters *AtSWEET2*, *AtSWEET4*, *AtSWEET14*, *AtSWEET16* y *AtSWEET17*. These data show how *Trichoderma* specifically influences the translocation of carbohydrates within the plant, which strengthen root exudation during a plant-fungus interaction.

079. Identification of mycorrhizal and rhizobial symbiosis specific iron transporter genes in bean

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Iron is an essential micronutrient for almost all living organisms, as it plays a fundamental role in metabolic processes such as DNA synthesis, respiration and photosynthesis. In addition, many metabolic pathways are activated by iron. In symbiosis, iron deficiency generally decreases the formation of nodules, hemoglobin production and nitrogenase activity, which leads to low concentrations of nitrogen in legume outbreaks. However, iron transporter genes have not been fully studied under mycorrhizae or nodulation conditions. In this work, we identify for the first time, the genes that participate in the transport of iron in beans. We identified 19 members from 5 different families such as NRAMP, YSL, VIT, IREG and IRT. Phylogenetic analysis reveals that these genes fit into 4 main clades. The analysis of the gene structure shows a greater number of exons in NRAMP and YSL compared to other families. Next, we perform the expression profiles of bean roots colonized with mycorrhizae or rhizobia. Among the 19 members, 12 correspond to genes involved symbiosis. Six unique genes were identified in each condition of mycorrhizal and rhizobial symbiosis. On the other hand, six genes shared between mycorrhiza and nodulation. Interestingly, YSL7 was highly regulated upwards (5 times) under nodulation conditions and YSL3 downwards (6 times) under mycorrhizal conditions. These putative genes could have an important function during bean symbiosis.
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080. *N-(p*-Coumaroyl)-L-homoserine lactone a quorum sensing signal produced by *Rhodopseudomonas palustris* modulate root system architecture in *Arabidopsis thaliana* through ethylene signaling pathway

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Many bacteria species communicate through use of small molecule signaling to modulate population density in a process denominated as quorum sensing (QS). *Rhodopseudomonas palustris* produces *N*-(*p*-Coumaroyl)-L-homoserine lactone to modulate QS system. Diverse Gram-negative bacteria interact with diverse plants in the rizosphere by using N-acyl-homoserine lactones (AHLs) modulating growth and defense in the plants. In the study we evaluated the effects of *N*-(*p*-Coumaryol)-L-HL on root system architecture in Arabidopsis. We found that *N*-(*p*-Coumaryol)-L-HL induces a dose-dependent effect on primary root growth, lateral root and root hair formation. This effects on primary root growth are related with an inhibition of mitotic activity in the primary root meristem by using reporter line *CyCB1::uidA* in Arabidopsis roots. Analyzing the molecular mechanisms we found that effects on primary root and lateral roots are independent of auxin signaling. Futhermore, we found that mutant related with ethylene signaling pathway *etr1-1, ein2-1* and *ein3-1* showed resistance to inhibition in the primary root growth, lateral root formation and root hair development. Our results, suggest that AHLs and *N*-(*p*-Coumaryol)-L-HL could share similar mechanism of signaling in plants.

081. Identification of Common Bean (*Phaseolus vulgaris)* Root/Nodule Symbiotic Mutants

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Legumes have the ability to establish symbiosis rhizobia, soil N-fixing bacteria, thus contributing to sustainable agriculture. Genetic resources such as insertion mutant populations from the model legumes *Medicago truncatula* and *Lotus japonicus* (*Tnt1* [Tagede et al. 2008] and *LORE1* [Urbanski et al. 2012] retrotransposon mutagenesis, respectively) have facilitated functional analysis of genes thus increasing knowledge about signalling, development, function of the legume-rhizobia symbiosis. Despite the agronomic importance of common bean (*Phaseolus vulgaris*), the most important legume for human consumption, to date we lack well characterized mutant population resources for this legume.

This work will present our progress towards the identification and characterization of common bean mutants affected in the root/nodule (R/N) symbiosis. We screened 1692 M4 lines from a mutant population developed by Porch et al. (2009) using the BAT 93 reference genotype and the EMS mutagen. For our screening, two seeds from each M4 line were inoculated with *Rhizobium etli* CE3, grown (watering with N-free solution) for *ca*. 25 days post inoculation, pulled up and visually checked for normal (wt) or altered (nod^- or nod^{++}) nodulation. A total of 93 candidate "nod^- mutant lines" were selected; these were re-planted and grown in fertilized conditions for obtaining seeds. The next step was to analyze the genetic segregation (wt:mut) of the mutant phenotype of the candidates. Ten M5 seeds from two plants of each candidate M4 "nod^- mutant line" were planted, inoculated and checked for nodulation. From these results we pursued the characterization of three main candidates that showed *ca*. 3:1 (wt:nod^-) segregation. We will present the results obtained from this analysis that included: histological characterization of initial symbiotic responses (root hair deformation, infection thread or nodule primordial formation), nodulation capacity with different rhizobia strains. Whole genome sequence of the 3 candidate mutant lines, that would allow mapping the mutated gene, is in progress.

082. The rhizospheric actinobacterium *Kocuria* sp. promotes root branching and biomass production in *Arabidopsis thaliana*.

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Bacterial communities are integral to the plant microbiome and reside in the rhizosphere, a soil region influenced by root exudation. Part of the fixed carbon during photosynthesis is transported to the root and secreted in the form of organic compounds that act as chemical attractans, and help establishing symbiotic relationships. Plant Growth Promoting Rhizobacteria (PGPR) produce metabolites that stimulate growth and enhance immune responses in horticultural species.
Although many efforts have been done applying microbes as bioinoculants in many crops, there is limited knowledge about the mechanisms that regulate plant growth promotion under bacterial symbiosis. Noteworthy, the actinobacteria group is prolific in production of bioactive secondary metabolites. In screens aimed at identify PGPR from rhizospheres of crops a *Kocuria* sp. isolate was typified based on molecular analysis. The aim of this work is to describe the growth promotion and defense-inducing properties of this bacterium in *Arabidopsis thaliana*. Our results show a strong phytostimulation in plants by direct interaction of roots with *Kocuria*, which enhanced primary root length, induced lateral root formation, chlorophyll content and fresh weight. This correlates with an enhanced auxin and cytokinin response. These data suggest that the actinobacteria may be an important component of the microbiome that determines growth and developmental traits for plant functioning in ecosystems.

083. Cloning of WRKY genes encoding transcription factors in banana and evaluation of their role in plant immunity

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Banana is a staple food for millions of people in Asia and Africa and a source of income in many Latin American countries through exports to U.S. and Europe. However, this crop is affected by fungal hemibiotrophic pathogens that cause partial or total loss of fruit production. The use of pesticides has been adopted as a conventional control measure; nevertheless, they have negative impacts on the environmental and human health. The genetic improvement of the current banana cultivars used for food security represents an attractive alternative to solve these problems since they would not require additional inputs to control the fungal pathogens. In this sense, the transcription factors of the WRKY family represent valuable alternatives for the genetic improvement of banana. The WRKY family of proteins play a key role in plant immunity by activating defense genes. Therefore, the aim of this work is to characterize four banana WRKY transcription factors named as MaWRKY18, MaWRKY45, MaWRKY60 and MaWRKY70 and to identify useful *MaWRKY* genes for banana breeding programs. So far, we have cloned the cDNAs of *MaWRKY18*, *MaWRKY45*, *MaWRKY60* and *MaWRKY70* and performed a structural analysis of these sequences. Moreover, we have determined the subcellular localization and transactivation activity of the proteins encoded by these *MaWRKY* genes. The role of these *MaWRKY* genes in plant immunity will be determined in the model plant *Nicotiana benthamiana* and banana.

084. Effect of two Actinobacterial strains, *Streptomyces ambofaciens* and *Streptomyces badius* on *Arabidopsis thaliana* growth

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The wide bacterial diversity and distribution in soil play essential roles in biogeochemical cycles and ecosystem functions. Actinobacteria are one of the most diverse and important Gram-positive bacteria in the soil, accounting for 10 to 33% of the total bacterial community. Many members of the Actinobacteria are able to solubilize phosphates, fix nitrogen, and produce different compounds such as siderophores, phytohormones, and antibiotics. These characteristics are desired in plant growth promoting bacteria, therefore improving plant development, photosynthetic health, and tolerance to biotic and abiotic stress.

Herein, we identified several Actinobacterial strains belonging to the genus *Streptomyces* using the 16S ribosomal gene. These strains were isolated from semiarid soils of San Luis Potosí state. Among them, we selected two strains in order to evaluate their effect on *Arabidopsis thaliana* plant growth *in vitro*. We determined differences in main root length, and root and rosette fresh weight. The plants inoculated with *Streptomyces ambofaciens* and *Streptomyces badius* showed an increase in root and rossete biomass, as well as a reduction in main root length. A notable increase in lateral root density was observed with *S. ambofaciens*. In order to elucidate some possible growth promotion mechanisms, auxin and cell cycle reporter lines of *A. thaliana* were used (*Tir1::GUS, CyCB1::GUS,* and *DR5::GUS*). We observed that both actinobacteria manage to stimulate the activity of the *GUS* reporter gene, which suggests that auxins are responsible for the growth promotion phenotype induced by these bacteria, as well, increments in cell proliferation in the root meristem.

085. Effect of a nanobactericide in tomato plants infected *Candidatus* Liberibacter Solanacearum transmitted by *Bactericera cockerelli*

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Candidatus Liberibacter solanacearum (*Ca*Lso), is a non-cultivable α-proteobacteria associated with the flower of plants belonging to the Solanaceae family and vectorized by the insect *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae). This pathogen induces so-called "permanent" diseases in tomato and "zebra chip" in potatoes, resulting in significant global economic losses. Since the diffusion of the bacteria, depends mainly on insects, the key to control this problem, is the vector. Unfortunately, this alternative turns out to be not very efficient, due to overuse of insecticides, which has triggered a strong resistance problem. Directly control the bacteria within the plant, can be inefficient mainly due to low nor access of the active ingredient used, to the area colonized by the bacteria, due to the characteristics of the molecule or the structure vascular it of the plant. As a result, this research focused on the evaluation of a nanobactericid (NB), i.e. spherical nanoparticles (90 mm in diameter), formed by the self-assembly of a copolimer in blocks, composed of a quaternized hydrophilic block and a hydrophobic acrylic block. Thanks to its nanostructured size, it is intended to improve its translocation within the plant and thus be driven to the specific sites where the pathogen is housed. For this purpose, bioassays were carried out in which the effect of NB was assessed, by means of a relative quantification of the pathogen, by the qPCR technique, the results relieved that the most effective dose was 70 ppm and decreased by 99.76% the bacterial load, vs the antibiotic oxytetracycline, which showed a 79.82% reduction under the same conditions, 21 days after infection.

086. Transcriptome analysis of resistant Mp717 and susceptible B73 maize inbred lines to *Fusarium graminearum* infection

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Maize is the most abundant cereal crop and has become a staple food in many parts of the world. It is one of the most important crops due to its multifunctionality as food, livestock, feed and biofuel production. However, one of the major challenges to global maize production is maize stalk rot caused by the hemibiotroph ascomycete *Fusarium graminearum* Schwabe, which is considered as major threat to production of maize accompanied by small losses to total wipeout of the crop. Maize infected with *F. graminearum* can be a severe threat to food safety for human and livestock consumption. The fungus produce mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA). Maize stalk rot symptoms include discolored from green to yellow or dark brown of the stalk. The fungus destroys the pith tissue causing diminishing water and photosynthate transport to leaves and ears, resulting in premature plant death. Commercial varieties are either intermediately resistant or susceptible to stalk rot. Breeding programs are focused on developing resistant maize cultivars. The maize inbred line Mp717 shows resistance to Fusarium stalk rot in contrast to the susceptible maize inbred line B73. In this study, we evaluate the differences in responses of maize inbred lines Mp717 and B73 infected with *F. graminearum* strain PH1 using RNA-seq. Differentially expressed genes (DEG) were identified between the susceptible B73 and resistant Mp717 maize inbred lines at 2, 14 and 28 days post infection (dpi). Gene Ontology categories were assigned to DEG and enriched terms were identified providing insights about the molecular mechanisms behind the response to *F. graminearum* infection in susceptible and tolerant maize inbred lines.

087. *In vitro* inhibition of phytopathogens with silver sulfide nanoparticles

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Phytopathogens are responsible for big economic losses worldwide. Some important plant pathogens include *Colletotrichum*, *Aspergillus and Clavibacter* species, which are pathogenic to various crops of great commercial importance for agriculture. Control methods for these pathogens are generally sources of environmental pollution, poisoning and other public health problems. At present, the use of silver nanoparticles has intensified, given their high antimicrobial capacity; however, some have been shown to present a risk to health and the environment. That is why recent research has focused on the study of silver sulfide nanoparticles (Ag2S-NPs), which have a very low solubility and greater stability. These characteristics mean that Ag2S-NPs could have environmentally friendly applications. In addition, there are reports that Ag2S-NPs present antimicrobial activity against pathogenic bacteria. Silver sulfide is a material that is present in the earth in three phases. The acantithe phase is the one that is stable at environmental temperature. In this work Ag2S-NPs at the acantithe phase are proposed as an alternative for the inhibition of phytopathogens. These nanoparticles have inhibitory and bactericidal properties. Furthermore, their synthesis is relatively cheap. We have synthesized nanoparticles using ultrasound waves and we have characterized them using electron transmission microscopy and X-rays. In order to evaluate the inhibitory potential we have tested them with the phytopathogens *Colletotrichum gloeosporioides*, *Aspergillus* spp and *Clavibacter michiganensis*. Our results in controlled laboratory conditions suggest a potential use as a control method in agriculture for the tested pathogens.

088. Global transcriptome analysis reveals the participation of MADS-box transcription factors in the establishment of legume-rhizobia symbiosis

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MADS-box transcription factors are the key regulators of various developmental processes in plants, such as flower development, detention of meristems, cell expansion and differentiation, shoot and growth, architecture and development. Unlike other plants, legumes have meristematic activity for a short duration while developing the root nodules. The contribution of MADS-box genes during the symbiotic interaction of legumes and rhizobium is not understood very well and the current studies are relevant to fill up the missing links in the symbiotic pathway in legumes. Herein, we have identified two subfamilies (type M and MICK) of the *Phaseolus vulgaris* MADS-box genes. A total of 49 members in M type and 43 members in MICK subfamilies were identified. The phylogenetic tree reveals 3 groups in each subfamily. The analysis of the gene structure of the MADS-box genes shows a minimum of 1 exon and a maximum of 8 exons. The protein domain analysis shows a conserved TF domain of the SRF type for all family members. In silico localization studies show that these proteins are located in the nucleus, mitochondria and chloroplast. In addition, the heat map shows the expression of these genes in different tissues and organs of plants. Our global transcriptomic analysis shows that under the rhizobial symbiosis conditions several members of the MADS-box gene family express differentially compared to the uninoculated roots. Among them, two genes of the MADS-box increased 7 times and 9 genes decreased 5 times. Based on our studies, we understand that these putative MADS-box genes could play an important role in the establishment and development of root nodule symbiosis in *P. vulgaris*.
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089. Effect of bacterial isolated from polluted soil by waste motor oil on root development of *Arabidopsis thaliana*.

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Plant growth-promoting rhizobacteria (PGPR) in some cases can grow in the soil and also to colonize root system plant, where are able to transform some products from the photosynthesis process into phytohormones for improving seed's germination and root nitrogen fertilizer (NIFE) uptake; either by biological fixing nitrogen (BFN) when this key element is not enough to cover plant's demand (Spaepen et al., 2014). PGPR can effectively provide this limiting element or through phytohormones to enhance NIFE uptake (Klama et al., 2010). In agriculture, applying PGPR in order to reduce and optimize NIFE is an important issue to avoid environmental pollution and preserve soil's fertility (Stefan et al., 2008). However, to understand how plant-microbial interactions result in plant health growth it's important for successful field applications (Farzana et al., 2009). According to this approach, *Arabidopsis thaliana* is utilized as a model system to elucidate plant microbiome and to determine mechanisms of plant interactions with PGPR (Zhao et al., 2018). The aim of this research was to analyze *Arabidopsis thaliana* responding to ARAB-1 and ARAB-2 and *Xanthobacter autotrophicus* at different levels of NIFE. In that sense, ARAB-1 and 2 were isolated from soil polluted by waste motor oil (WMO) and were inoculated at seed and seedling stage of *A. thaliana* to observe seed's germination and root development in different NIFE source concentration (0%, 50%, 100%). Then three strategies were applied: 5-day-old seedlings transference to inoculated media, direct sowing in inoculated media and seed biopriming and sowing in non-inoculated media. Authors appreciate the support of Proyecto 2.7 CIC-UMSNH (2019) and Harvard University Rockefeller Fund, Boston, Mass, USA (2019). Farzana Y., Saad R. O. S. & Kamaruzaman S. (2009). *Austr. J. Basic Appl. Sci.* 3:1641-1644. Klama J., Wolna-Maruwka A. & Niewiadomska A. (2010). *Nauka Przyr. Technol*. 6:1-7. Stefan M., Mihasan M. & Dunca S. (2008). Scientific Annuals of University "Alexandru Ioan Cuza" Iasi, *Sect. Genet. Mol. Biol. T*. IX, 3:105-110. Spaepen S., Bossuyt S., Engelen K., Marchal K. & Vanderleyden J. 2014. New Phytol. 201: 850-861. Zhao C. Z., Huang J., Gyaneshwar P. & Zhao D. (2018). *Front. Microbiol.* 8:2556

090. Chemical profiling of the exo-metabolome of *Fusarium kuroshium*, causal agent of the plant disease Fusarium dieback[£]

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Fusarium kuroshium is a phytopathogenic fungus that establishes a symbiotic relationship with the ambrosia beetle *Euwallacea kuroshio* (Kuroshio shot hole borer). Currently, this insect-microorganism complex is an exotic pest that is dispersed in the state of California (USA) and since 2015 there are reports of its presence in Tijuana, Mexico. *F. kuroshium* along with *Graphium* sp. are the causal agents of a plant disease named Fusarium dieback, which known hosts include both agricultural and forestry importance, more than 200 plants in total. Since the ecological and economical negative impacts of this microorganism and the lack of information on the produced metabolites that may be involve in its phytopathogenic effect, the main goal of this work was to characterize the secreted molecules by this fungus and preliminary elucidate their role in the infection process. For this, organic fractions obtained in EtOAc, n-ButOH and ACN:H_2O from *F. kuroshium* cultured *in vitro* were analyzed by untargeted and targeted metabolomics approaches based on accurate and tandem mass spectrometry, respectively. Among the results, fonsecin, T2-triol toxin, flaviolin and some other toxins were putatively identified by the untargeted analysis along with more than 60 different *m/z* ratio values which still unidentified. In addition, the presence of fusaric acid was confirmed by the targeted analysis in the EtOAc fraction (0.36 ± 0.05 µgL-1), and when this compound was tested (8.92 mM) on an *in vitro* leaves discs system using an economical important host species such as *Persea americana* Mill. (Lauraceae), a total necrotic damage was observed on the foliar tissue. Furthermore, only EtOAc fraction at 1.6 $mgmL^{-1}$ caused visible damage to *P. americana* leave discs when all organic fractions were tested. This work is the first report that describes the chemical arsenal of *F. kuroshium* and its plausible role in the phytotoxicity of this fungus. This research was supported by the Project#291399 of Fondo Institucional de Fomento Regional para el Desarrollo Científico, Tecnológico y de Innovación (FORDECYT)-CONACyT.

091. Rhizobia exopolysaccharide-mediated evasion of host immune defense response during symbiotic nitrogen fixation

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The application of synthetic nitrogen fertilizer has helped improve crop yield. However, leaching of excess fertilizer can devastate surrounding aquatic ecosystems. Legumes overcome this macromolecule limitation by forming a symbiosis with soil microbes, or rhizobia, which produce usable nitrogen in root structures called nodules. To combat the negative effects of fertilizer overuse, the molecular dialog that establishes symbiosis has been studied extensively. The method by which rhizobia evade the host innate immune response is essential for symbiosis but is not fully understood. Using the rhizobium species *Sinorhizobium meliloti* and the legume *Medicago truncatula* as our model system, we explore the role of rhizobial exopolysaccharide (EPS) in defense evasion during symbiosis. First, microbial exopolysaccharides in general have been noted for their protective properties during host interactions. Here, we will address this property by overproducing EPS in *S. meliloti* strains and test whether these strains can survive in host nodules that exhibit increased immune defense responses. Furthermore, we will address a more direct role of EPS in the evasion of host defenses. *M. truncatula* contains two Lysin motif-containing proteins (LYM1 and LYM2) that trigger defense responses upon binding pathogen associated molecular patterns (PAMPs), such as peptidoglycan. Previous studies in our group have shown that if the two LYM proteins are over-expressed in roots inoculated with *S. meliloti*, the symbiotic infection weakens due to increased activation of host defenses. Here, we will overexpress LYM1 and LYM2 using the hairy root transformation method and test if EPS overproduction attenuates plant defenses by interfering with LYM detection of PAMPs. We will assess the viability of rhizobia within nodules by using confocal imaging and determining colony forming units. This work will inform the protective role of rhizobial EPS in the context of host defense evasion during symbiosis.

092. Role of the AON process in the establishment of the legume-rhizobia symbiosis under phosphate deficiency conditions

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Phosphate (Pi) deficiency reduces nodule formation and development in different legume species. Despite the significant progress in the understanding of the genetic responses underlying the adaptation of nodules to Pi deficiency, it is still unclear how this nutritional condition reduces the nodule number in different legumes, including common bean. We recently demonstrated that Pi deficiency affects the molecular dialogue between common bean and rhizobia. Likewise, we demonstrated that regardless of the presence or absence of rhizobia, the expression of *PvRIC1* and *PvRIC2*, two genes participating in the Autoregulation of Nodule Number (AON), was always higher in Pi-deficient common bean seedlings than in control seedlings. This data clearly indicates that AON might play a role in the establishment of the legume-rhizobia symbiosis under Pi-deficiency conditions. Here, through grafting experiments on both common bean and soybean *nark* mutant plants, we provide evidence indicating that Pi deficiency activates the AON process in the absence of rhizobia. Finally, we propose that Pi deficiency activates the AON process prior to the interaction with rhizobia.

093. *Achromobacter* sp. 5B1, a rhizobacterium isolated from an extreme environment, influences directional root growth through auxin signaling and redistribution in *Arabidopsis*.

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Roots provide physical and nutritional support to aboveground plant organs and play critical roles for adaptation via intricate movements and growth patterns. Through screening the effects of bacterial isolates from roots of halophyte Mesquite (*Prosopis* sp.) in *Arabidopsis thaliana*, we identified *Achromobacter* sp. 5B1 as a probiotic bacterium with novel and emerging functional properties. Detailed genetic and architectural analyses in *Arabidopsis* grown in vitro and in soil, cell division measurements, and confocal analysis of auxin transport and response genes demonstrated that root colonization with *Achromobacter* sp. 5B1 changes growth and branching patterns via root tip navigation, which was related to auxin perception and redistribution. A rapid redistribution of auxin within the primary root tip of wild-type seedlings by *Achromobacter* sp. 5B1 correlates with repression of auxin transporters *PIN1*, *PIN2* and *PIN7*, whereas in seedlings harboring *AUX1*, *EIR1*, *SLR1*, *AXR1*, *ARF7ARF19*, *TIR1AFB2AFB3* single, double or triple loss-of-function mutations, the bacterium caused primary roots to form supercoils that are devoid of lateral roots. Thus, *Achromobacter* sp. 5B1 fine tunes both root movements and the auxin response, and defines a novel mode in the regulation of root behavior by the bacterial microbiome.

094. Effect of arbuscular mycorrhiza colonization on the susceptibility to herbicides in tomato

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Herbicides are the most used pesticides worldwide because of their effectivity on weed control. In Mexico, the use of herbicide in tomato crops is a common practice. Although herbicide targets are weed plants, it is common that crop plants are negatively affected. Since mycorrhiza colonization bring benefits to crop plants such as reduction on susceptibility against biotic and abiotic stresses, it has been hypothesized that this symbiosis induced certain resistance to the damage caused by herbicides. Then, the objective of this work was to study the effect of mycorrhiza colonization on the alleviation of the damage caused by two herbicides on tomato plants, as well as on the induction of defense by the establishment of this symbiosis. A dose response assay of glyphosate (0, 1, 2, 4, 6, 8 y 10 L/Ha) and paraquat (0, 0.75, 1.5, 2, 3, 4 y 6 L/Ha) on tomato growth was evaluated on 8 week-old plantlets, and it was demonstrated that biomass accumulation is affected by both herbicides, even at the lowest concentration tested. When mycorrhizal plants were exposed to glyphosate (4 L/Ha) and paraquat (0.75 L/Ha), they did not show any alleviation on the damage cause by the herbicides on the non-mycorrhizal control, which indicates that the mechanism that induced resistance to other stress do not apply to herbicide. On the other hand, mycorrhiza induced resistance was manifested in colonized plants even under glyphosate treatment, indicating that biosynthesis of aromatic amino acids are not involved in the mechanisms of mycorrhiza induced resistance. Paraquat treated plants, on the other hand, showed no induction of defense in mycorrhizal plants, which indicates that this herbicide blocks the onset of the induced resistance. Expression of several markers gene of jasmonate biosynthesis are currently analyzed in herbicide-treated plants, in order to explore the possibility that jasmonates are involved in the response mechanism. (SIP-IPN 20196531).

095. Transcriptional analysis of mycorrhiza induced genes related to cell wall and cuticle formation

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Arbuscular mycorrhizal association provides nutritional benefits to plants. Additionally, it induces a physiological state that allows plants to response in a more rapid and intense manner to a subsequent biotic attack; consequently, mycorrhizal plants become less susceptible to both root, as well as shoot pathogens. Although, it has been suggested that most molecular changes induced by mycorrhiza colonization in shoots might occur at the posttranscriptional level, it has been documented that transcriptional changes also occur (Cervantes-Gamez et al., 2016). Possible mechanisms that can explain, in part, mycorrhiza induced defense, is the reinforcement of cell walls through accumulation of cellulose, and upregulation of the expression of a cellulose synthase gene (Rodríguez Corral, 2019), as well as cuticle modification. The aim of the present work was to identify and characterize candidate genes differentially regulated in shoots of mycorrhiza colonized *vs* non-colonized tomato plants with *Rhizophagus irregularis*, which could be involved in the reinforcement and/or biogenesis of cell walls, as well as genes potentially related to cuticle formation. Three genes related to cell wall metabolism, and two to cuticle formation were analyzed by qPCR and all of them showed upregulation at the transcriptional level. These results support the hypothesis that reinforcement of cell wall and cuticle, which have already been associated to defense against pathogens (Ziv et al., 2018), play a role in the priming mechanism triggered by mycorrhiza colonization by strengthen the first physical barriers for pathogens. (SIP-IPN 20196531).

096. Spore-based formulation from *Bacillus thuringensis* TA26 and *B. subtilis* TA16 antagonistic strains against *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 3 from tomato.

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Tomatoes are the second most important vegetable worldwide. Mexico occupies the tenth place in tomato world production. Tomato cultivation can be affected by different factors - which limit its production and profitability – such as the diseases caused by the fungi *Fusarium oxysporum* f. sp*. lycopersici* (*Fol*) that causes the tomato vascular wilt leading to yield losses of more than 50%. Three *Fol* races have been described, being race 3 the most devastating one.
The use of biological control agents (BCA) is a good alternative for pest and disease management. Bacteria belonging to the genus *Bacillus* stand out for their mechanisms of antagonism to fungal pathogens, and their ability to produce endospores, which allows creating biofungicide formulations with a long shelf life. The objective of this work is to obtain spore formulations of two *Bacillus* strains antagonistic against *Fol* race 3 both *in vitro* and *in planta*, with potential use as a biological product for the inoculation of tomato seeds and for disease prevention in the field.
Three different sporulation media were evaluated, selecting DSM medium (Difco Sporulation Medium) which allowed the highest spore production (8.52 and 6.12 $x10^8$ spores/mL) for TA16 and TA26 respectively, after 72 hours of fermentation. Spore-based powder formulation was obtained for both strains. Formulation process viability was 67% and 70% for TA16 and TA26, respectively. Fermentation parameters for spore production including temperature, stirring speed and pH were optimized, under a factorial design 2^3. The best conditions for spore production were similar for both strains: 35 °C, 150 rpm and pH 5.6. Spore viability for TA16 and TA26 powder formulations has been stable during the first six months of storage at room temperature (25 °C) remaining with 90 and 77% spore viability for TA16 and TA26, respectively. Both strains in spore-powder formulation maintained their antagonistic activity against *Fol* race 3 both *in vitro* and *in planta*.

097. *Bacillus velezensis* 83 as a stimulator of the systemic defenses of *Arabidopsis thaliana*

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Bacillus velezensis 83 is the active ingredient in the product Fungifree AB®. Its effect on the field has been demonstrated in various crops, however, the mechanisms by which this bacterium is capable of exerting a biological control effect have not been studied in detail. This study analyzes the effect as a stimulant of the systemic defenses in *Arabidopsis thaliana*, against the infection by the necrotrophic fungus *Botrytis cinerea*. This was evaluated in an *in vitro* culture system where the root system is in direct contact with the bacteria or bacteria metabolites, ensuring no physical contact with the leaves of the plant where the pathogen is applied. We found that *Bacillus velezensis* 83 is an Induced Systemic Resistance effect, which decreases the incidence of infection to less than 50%. This effect changes in relation to the concentration of the inoculum applied. Interestingly, the application of bacterial supernatants obtained by fermentation in rich medium, were also able to reduce the disease incidence to 20%. This suggests that the bacterium has the metabolic potential to synthesize stimulatory compounds of plant defenses, however, its sole presence in the rhizosphere is not sufficient to exert an efficient effect. This study opens the way to understand how to enrich the bacterial content with suitable metabolites in order to induce an effective systemic resistance effect against economically important pathogens.

098. *In vivo* visualization of the cell plate formation at the rhizobia-infection site in *Phaseolus vulgaris* roots

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Cell plate (CP) formation during cytokinesis is a plant cell specific process. The CP is formed *de novo* as a tubulo-vesicular network originated at the center of the cell-division plane, it grows radially to fuse to the parental plasma membrane (PM), leading to separation of daughter cells. The CP is generated by the targeting and fusion of Golgi-derived vesicles carrying the components of the new PM and cell wall. Such process depends on the plant cytokinesis-specific syntaxin KNOLLE, a useful molecular marker to study plant cytokinesis. KNOLLE gene is transcribed specifically in the G2 phase of the cell cycle, whereas the protein is synthesized during M phase[1]. At the end of cytokinesis, KNOLLE is targeted to the vacuole for degradation[2]. In the legume:rhizobia symbiosis (nodulation), the first step of the infection process takes place when the rhizobial-signalling molecules, known as Nod factors, are perceived by a receptive growing root hair. Such a molecular dialogue, leads to the formation of a transcelullar tunnel or infection thread (IT), through which rhizobia get access to the root cortical zone. In parallel, the cell cycle of root cortical cells is re-activated to form a nodule primordium. In *Phaseolus vulgaris* nodulation, the cortical cells adjacent to the root hair forming an IT are the first to divide. In order to easily visualize the dynamics of IT formation and cell division, we have generated *P. vulgaris* transgenic roots bearing the cassette prom*Pv*KNOLLE:YFP-*Pv*KNOLLE. To track the IT formation, transgenic roots were inoculated with *Rhizobium etli* CE3-pMP604 expressing DsRed. As predicted, confocal images revealed that YFP-*Pv*KNOLLE was restricted to dividing cells in the root apical meristem, in the lateral root primordia, as well as in the cells adjacent to the infection site. The subcellular distribution of YFP-*Pv*KNOLLE was potentially associated to the CP. We are currently monitoring the time lapse between the IT formation/elongation through the root hair and the CP expansion.

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099. Virus induced gene silencing (VIGS) as genetic tool to control *Fusarium solani*

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The fungal pathogens are an important group of phytopathogens that threats crops of commercial interest, which it causes significant losses in crop yield and merchandising in the world. Among fungi that affect plants, ambrosial fungi represent a huge problem due to their complex interaction that involves: the host plant, the vector insect and the ambrosial fungi. This complex interaction limits the control of these pests. A strategy that has been proposed as a control method is the use of RNA silencing, a mechanism that involves the suppression of genes at the transcriptional or post-transcriptional level, and that offers new opportunities for the development of beneficial applications for agriculture. On the other hand the virus-induced gene silencing (VIGS) is a tool that has allowed the analysis and characterization of the function of different genes in plants, but also offers an alternative for the fight against pathogens by designing vectors with objective genes of the fungus, avoiding the generation of transgenic plants. In this study we focus on the search of target genes against an ambrosial fungus and the construction of a VIGS vector that allows analyzing the role of these genes in the spread of the fungus.
For the construction of the VIGS vector, we selected the chitin synthase (CH1-7) gene family. This is a group of enzymes that are important in the synthesis of chitin, a structural component of the cell wall and these enzymes are also relates to the spread of hyphae. The primers were constructed using 14 sequences of filamentous fungi related to the clade of *Fusarium solan*i, available in the NCBI Gene Bank. A 214 bp fragment was obtained by PCR, and it was cloned in the VIGs vector *Euphorbia Mosaic Virus*-Yucatan Peninsula-CP-Chi. This VIGS vector will be tested in *Nicotiana benthamiana* plants infected with *Fusarium solani* in order to know the role of chitin synthase silencing gene in the plant resistance to this fungi.

100. Genome-wide identification of RWP-RK transcription factor family genes in nodulated roots of bean

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RWP-RKs represent a small family of transcription factors (TFs) that are unique to plants and function particularly under conditions of nitrogen starvation. These RWP-RKs have been classified in two sub-families such as, NLPs (NIN-like proteins) and RKDs (RWP-RK domain proteins). In *Arabidopsis*, NLPs functions in nitrogen use efficiency whereas, RKDs regulate gametogenesis/embryogenesis and some unknown functions. So far, little is known about the role of RWP-RKs in legumes specifically, during mycorrhizal and rhizobial symbiotic interaction. Herein, we identified nine RWP-RK TF family members in bean (*Phaseolus vulgaris*), among them 7 were NLPs and 2 were RKDs. These TFs were equally dispersed on seven chromosomes except the chr9 which show 3 members. Ten alternative transcripts were found for all the NLPs and in contrary, RKDs did not have any alternative transcripts. RWP-RK domain was well conserved among all the members whereas, PB1 domain was restricted to NLPs alone. Further, the RTqPCR analysis shows that during rhizobial symbiosis, most of the NLPs upregulated among which NLP4 and NLP6 was significantly increased relative to control. On the other hand, during mycorrhizal colonization, RKD1, RKD2 and NLP5 genes upregulated compared to controls. Interestingly, RKDs were very specific to mycorrhizal symbiosis whereas, NLPs for nodulation. Based on the expression analysis our results suggests that the members of both the sub-families of RWP-RKs may have putative role in establishing the rhizobial and mycorrhizal symbiosis in bean. Nevertheless, more experimental evidences are needed to unravel the putative role of these TFs in the model legume, bean. This work was supported by PAPIIT (DGAPA-UNAM) grant no. IA207219 to MK.A and IN211218 to K.N.

101. Genetic comparison of the microbiome between asymptomatic and symptomatic plants of *Vanilla planifolia* affected with the root and stem rot disease

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The vanilla plant (*Vanilla planifolia*) is susceptible to fungal agents that cause root and stem rot disease. This disease is associated with the genus *Fusarium*, in particular the species *Fusarium oxysporum*, which is often identified as the main causative agent of the disease. However, the isolation of these fungi from symptomatic plants, as well as asymptomatic plants and wild vanilla indicates that these fungi are naturally associated with vanilla and that their pathogenicity is triggered by various other factors. Our genetic characterization using genome-wide amplicons of 16SrRNA (bacterial) and ITS (fungal) suggests that the diversity of endophytic microorganisms are important to promote or inhibit the development of the disease. Although we did not find differences between the overall diversity compositions of the microbiomes of these groups, we find differences in the presence and abundance of certain taxa, including those with potentially inhibitory properties of *Fusarium oxysporum.* We propose that cause root and stem rot disease is influenced by the interactions of the plant with other endophytic microorganisms that can favor or inhibit the development of the disease.

102. Plant Nitrogen Network (PlaNNet) - Coordinating Research on Plant Nitrogen for Sustainable and Productive Agriculture

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PlaNNet aims to coordinate research activities related to the uptake and utilization of N by plants with the long-term objective of enhancing the efficiency and sustainability of N-use in agriculture. PlaNNet has connected basic and applied researchers to facilitate efforts to solve agriculture's N-problems. RCN activities, coordinated by a Steering Committee, include: (i) a networking website that presents information about researchers around the world who are involved in plant N-related research, (ii) Workshops-Without-Walls (WWW) that engage participants in presentations and discussions, related to plant-N research; and (iii) satellite workshops at major conferences that focus on aspects of plant N and agriculture.
Presentations at conferences and virtual workshops describing the rationale for PlaNNet and current R&D strategies to solve problems related to N over-use or inadequacy in agricultural systems have informed and connected groups conducting N-related research around the world, including *academic, industry, agriculture, and environmental groups.*

The PlaNNet website (http://plannet-rcn.org/) presents information about scientists involved in N-research and their various projects, information related to N-related R&D, including funding and collaboration opportunities.

103. Heterologous expression of an extracellular tetraspanin domain from *Marchantia polymorpha* and generation of a specific antisera in rabbits

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Generation of reactive oxygen species (ROS) is a key process during pathogenic and mutualistic interactions. During mutualistic interactions between *Phaseolus vulgaris* and beneficial microorganisms such as rhizobacteria and arbuscular mycorrhizal fungi, the role of ROS producing enzymes such as NADPH oxidases have been widely analyzed. Tetraspanins are transmembrane proteins that have been suggested to be part of a mechanism that generates ROS; in addition, we have reported the spatiotemporal expression of these genes during the plant–rhizobacteria and plant-mycorrhizal fungi interactions. The generation of gene knockout or knockdown in *P. vulgaris* is very difficult since these plants are recalcitrant for stable transformation, and thus, we are limited to the generation of composite plants. Furthermore, the number of tetraspanins related copies in common bean is at least 13 members. In order to gain insight into the general role of tetraspanins in plants, we focused on the model plant *Marchanthia polymorpha*, which has only two tetraspanin genes and is amenable for gene editing and stable transformation. We are interested in characterizing the expression pattern and protein level. In order to address the protein characterization, we must generate specific antisera to detect the tetraspanins in protein extracts in different tissues or developmental stages. The tetraspanin cDNA fragments coding for the large extracellular loops were PCR amplified and sub-cloned into an expression vector to be expressed in *Escherichia coli* as 6XHis tagged polypeptides. The recombinant proteins will be purified by immobilized-metal affinity chromatography, emulsified with adjuvant, and then injected to rabbits by intradermal and subcutaneous routes. Thereafter, the antisera will be monitored against recombinant proteins until a good title is reached. The specific antisera will be employed to monitor the tetraspanin accumulation in different tissues or different developmental stages.

This work was supported by DGAPA IN-209118 and CV200519 and Scholarship No. 314168 from CONACYT to SJJ.

104. *Pseudomonas putida* and *Pseudomonas fluorescens* influence Arabidopsis root system architecture through an auxin response mediated by bioactive cyclodipeptides.

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Plant growth-promoting rhizobacteria modulate root development through different mechanisms. This work was conducted to evaluate the effects of root colonization by *Pseudomonas putida* and *Pseudomonas fluorescens* in biomass production, lateral root formation, and activation of auxin signaling in *Arabidopsis thaliana.* Selected strains of *P. putida* and *P. fluorescens* were tested for modification of *DR5::uidA*, *BA3::uidA* and *HS::AXR3NT-GUS* auxin-related gene expression, and to promote root hair and lateral root formation in WT and *tir1-1, tir1-1afb2-1afb3-1, arf7-1, arf19-1, arf7-1arf19-1*, and *rhd6* mutants. Production of cyclodipeptides with possible roles in auxin signaling was also determined in *P. putida* and *P. fluorescens* culture supernatants by gas chromatography–mass spectrometry. *P. putida* and *P. fluorescens* stimulated lateral root and root hair formation and increased plant biomass, which correlated with an induction of the auxin response. Genetic analyses suggested that growth promotion involves auxin signaling as *tir1-1, tir1-1afb2-1afb3-1, arf7-1, arf19-1, and arf7-1arf19-1* mutants showed decreased lateral root response to inoculation and because *P. putida* and *P. fluorescens* restored root hair development in the *rhd6* mutant. It was also found that these bacteria produce the cyclodipeptides cyclo(L-Pro-LVal), cyclo(L-Pro-L-Phe), and cyclo(L-Pro-L-Tyr), which modulates auxin-responsive gene expression in roots. Our results suggest a role of cyclodipeptides for bacterial phytostimulation.

105. Stimulation of growth and secondary metabolism in *Stevia rebaudiana* by *Enterobacter hormaechei,* an endophytic bacterium

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Stevia rebaudiana Bertoni is a plant that accumulates steviol glucosides (SGs) and phenolic compounds in their leaves. SGs are a natural non-caloric sweetener commercially demanded by the overweight and diabetes population. Consequently, there is interest to use microorganism with growth promoting activity to improve crop yields. Endophytic bacteria could be excellent candidates, because they are microorganisms that live within the plant tissue in a mutualistic interaction. Bacteria promote growth through the secretion of auxins and gibberellins and can stimulate secondary metabolism. The objective of this study was to evaluate the activity of 12 endophytic strains isolated from *S. rebaudiana* plants in the plant growth promotion and accumulation of their secondary metabolites. The plants were harvested 30 days after inoculation, determining growth parameters (plant height, number of leaves and root length), and secondary metabolite content (SGs, phenolic compounds and flavonoids). *Enterobacter hormaechei* Lb1 isolated from roots was noted for its ability to stimulate root growth 1.33 folds more than the control plants. While, *E. hormaechei* 1017 isolated from leaf was unleashed by promoting an increase in the content of SGs in the leaves 2.2, phenolic compounds 1, 32, and flavonoid contends 1.43 times, compared to plants without inoculation. These results indicated that *E. hormaechei* Lb1 and *E. hormaechei* 1017 may be used to improve *S. rebaudiana* yields. The autors acknowledge to CONACYT for the scholarship (702975) and Instituto Politécnico Nacional (Grant SIP20195064).

106. *Hanseniaspora opuntiae* is a biostimulant of *Arabidopsis thaliana*

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We have recently shown that compounds released by the biocontrol yeast *Hanseniaspora opuntiae* can protect soybean (*Glycine max*) and *Arabidopsis thaliana* plants against the broad host-range necrotrophic fungi *Corynespora cassiicola* and *Botrytis cinerea* , respectively. To evaluate whether *H. opuntiae* could have another biostimulant effects acting directly on the plant, *A. thaliana* plants were grown *in vitro* in MS agar plates which contained a distant inoculum of *H. opuntiae* . After seven days, principal root length was shorter and the number of lateral roots was notably higher in plants grown in plates with the inoculum. Fresh weight was also higher in these plants compared with the control. Similar phenotypes have been described for the interactions of plants with *Trichoderma sp* . fungi, where secondary metabolites secreted by fungi affect hormone signaling in plants. Given that lateral root growth and biomass increase are mediated by phytohormones and that auxin secretion by *H. opuntiae* has been reported, to test if the responses in *A. thaliana* are mediated by hormone signaling, plant auxin and ethylene reporter *A. thaliana* lines were used, and the results confirmed this hypothesis. Some fungi exert their biostimulant effects via volatile compounds without having physical contact with the plants. For this reason, *A. thaliana* was grown in split plates with or without yeast, where plants were placed in one of the compartments of the plate and in the yeast in the other, avoiding physical contact and possible compound diffusion by agar. Plants grown in split plates with yeast also presented the already mentioned phenotypes. These results indicate that *H. opuntiae* yeast not only has a biocontrol effect against pathogen fungi but also has a biostimulant effect in *A. thaliana*, reorganizing the roots architecture and with an increase in biomass.

107. Characterization of microRNAs and mRNAs expression profiles in avocado (*Persea americana* var. *drymifolia*) during an ambrosia *Fusarium solani* infection

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Mexico is the main producer of avocado (*Persea americana*) in the world, with an estimated production of 1.9 million metric tons per year. The implementation of phytosanitary pest-control programs in this crop is a priority to reduce losses. Fungal diseases are the main concern in avocado production, and currently, *Fusarium*-dieback, an emerging disease is the focus of a national control program. It is caused by *Fusarium kuroshium* and *Fusarium euwallaceae,* symbionts of ambrosia beetles. Despite interest in this species, the molecular mechanisms associated with the disease remain unknown. In this work, we used an ambrosia *Fusarium solani strain* isolated from *Xylosandrus morigerus*, a native ambrosia beetle to infect avocado (*P. americana* var. *drymifolia)* seedlings through their roots under hydroponic conditions, in order to characterize the phenotypic and transcriptional responses including expression of microRNAs. At 21 days post-infection (dpi) typical symptoms of fusariosis, such as necrosis and foliar tissue decoloration were evident. mRNAs and microRNAs libraries were generated at 1, 7, 14 and 21 dpi from *F. solani*-infected and not infected roots. A *de novo* wide coverage transcriptome was assembled. A total of 285,867 unigenes were annotated and 11,851 differentially expressed genes were identified. We found a clear transcriptional reprogramming process in late stages of infection, where the overexpression of unigenes encoding pathogenesis-related proteins and key enzymes of phytohormones biosynthesis occur. We found 146 genes that encode R proteins involved in the defense process. Additionally, 384 miRNAs were identified in response to infection. Interestingly, we did not find any miRNAs present in all the infection time-points. Also, microRNAs have higher presence in late times of infection, concluding that transcriptional response is critical after 14 days post infection. This work identified for the first time avocado miRNAs during an ambrosia fungus infection.

108. Emerging role for the tetraspanins in *Phaseolus vulgaris* and their connection with mutualistic interaction

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Root hairs are tubular extensions from root epidermal cells which experiment polarized tip growth. This polar growth occurs at the apex of the cell and involve the fluxes of ions, cytoskeleton rearrangements and exocytosis of secretory vesicles. It has been suggested that the activity of the NADPH oxidase and the reactive oxygen production are key players for polar growth. In fact, the NADPH oxidase is highly localized in membrane microdomains at the growing tip. In this work we have addressed the exploration of a new family of protein called “tetraspanin” which have emerged as a new membrane component able to organize as tetraspanin enriched microdomain or web in the plasma membrane. These proteins have been broadly studied in human cells, nematodes, fungi and flies, however, in plants is very limited. Tetraspanins are integral membrane proteins with two extracellular loops containing several highly conserved cysteine residues. These loops allow their association to microdomain forming clusters in different levels of complexity. Tetraspanins also contributes to exosome formation, which are extracellular vesicles derived from the multivesicular body that carry DNA, mRNA, microRNA, proteins and lipids. Thus, the key role for exosomes is the intercellular and interkingdom communication. We have addressed the phylogenetic, subcellular localization and promotor activity for some of the tetraspanins in the legume *Phaseolus vulgaris* during mutualistic interactions. We found that all the sequences encoding tetraspanins maintained the classical signature GCC-(K/R)P, a motif that define the tetraspanin family. By transcript accumulation analysis and promotor activity, we found a differential expression in different tissues such as: primary root, root hairs, lateral root and nodule primordia from *P. vulgaris* induced by rhizobia. These results, including the subcellular localization of tetraspanins, will be presented and discussed.
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109. Functional characterization of a LORELEI gene during the nodulation process

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In plant cells, reactive oxygen species (ROS) play an important role in several physiological processes, for example: in plant development, hormonal signaling, polar growth, biotic and abiotic interactions, etc. It is well known that EROs represent a dual role during stress and development processes, it means that the concentration, dynamics and subcellular distribution could have antagonistic responses in a similar way to intracellular calcium. In plant cells, the biogenesis of ROS has been widely linked to the NADPH oxidase activity. However, the activity of NADPH oxidase depends from other molecular interactors acting upstream the signaling cascade. For instance, FERONIA (a receptor like kinase) has emerged as an important regulator for the NADPH oxidase, which regulates the localized ROS production at the tip of pollen tubes and root hairs. In root hairs, both FER and NADPH oxidases are localized in the apex of the growing cells, where lipid rafts domains have been described. These membrane microdomain are sterols enriched regions and contains some proteins modified with a glycosyl-phosphatidyl-inositol (GPI). In Arabidopsis thaliana, mutation in llg1-2 a LORELEI like protein (LGPI) which has a GPI motif results in a phenotype very similar to fer4 and affects the targeting of FER to the plasma membrane and thus FER is retained in the endoplasmic reticulum. This result in a clear decreased ROS production. Since FER requires LGPI protein for a correct targeting to the plasma membrane, we have functionally analyzed the LGPI in Phaseolus vulgaris and evaluate their role during the mutualistic interaction with Rhizobium tropici. We have characterized at least one LGPI gene and found that the transcript significantly accumulates in mature root nodules. Furthermore, the promotor activity is also high in mature nodules, suggesting a functional role during the nodule development or function. The functional analysis of LGPI during the P. vulgaris-Rhizobia interaction was addressed by silencing or overexpressing the LGPI gene in composite plants. These results will be presented and discussed.

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110. Effect of phytohormones on the mycorrhizal association of the *Metarhizium* fungus

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No living organism has an exclusive ecological niche. All organisms form a network of interaction with other organisms; charities, competition, parasitism, among others. In the rhizosphere, plants have created complex networks with different microorganisms. In these complex networks the plants release metabolites, which can help the plant to defend itself against damage by phytopathogenic microorganisms, they also use these metabolites to attract beneficial organisms, such as Rhizobacteria and fungi, however, these chemical signals can also be detected by pathogens of plants, both bacteria, fungi and plants. These chemical compounds include oligosaccharides, such as glucan and chitin, polysaccharides like pectin, other molecules such as glycoproteins, lipids, toxins, effector proteins and phytohormones. One of the most important are the beneficial associations of fungal plants, they are called mycorrhizae. The mycorrhizal associations are old and widely distributed, occur above 80% of land plants. In this symbiosis the fungus acts as an extension of the roots of the plant, increasing the surface area for nutrient intake, and the fungus facilitates the solubility of compounds such as phosphates. On the other hand, plants provide the fungus with energy in the form of fatty acids, sugars, vitamins, amino acids among other low molecular weight compounds. In mycorrhizal associations, chemical signaling between them is important, from the beginning of the association until its establishment. As mentioned earlier, phytohormones are a class of compounds that the plant uses to seek establishment with mycorrhizal fungi. Phytohormones are released to the soil through the roots, play an important role in mycorrhizal associations, in arbuscular mycorrhizal fungi, some phytohormones such as stringolactones and geberellic acids promotes mycorrhizal association, increases the number of branching of hyphae and in phytopathogenic fungi promotes the germination of the spores.In the working group the relationship of the *Metarhizium* fungus with plants is studied, which is a mycorrhizal entomopathogenic fungus, this fungus colonizes the root of the plants as an ectomycorrhiza and transfers the nitrogen from insects that it has infected to the plants, also produces plant growth promoting compounds; for instance, auxin, 3-indoletic acid. In 2017, Davila-Berumen, isolated a strain of *Metarhizium guizhouense* with antagonistic capacity against *Fusarium oxysporum* f sp. Lycopersici, in dual confrontations formed a halo of inhibition and in tripartite interactions.

111. Silencing of *AGO5* in transgenic roots of *Glycine max* (soybean) using CRISPR-Cas9 technology

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Legumes are able to fix atmospheric nitrogen through the symbiosis with rhizobia. This symbiosis is regulated at the transcriptional, posttranscriptional, and posttranslational level. It has been suggested that Argonaute (AGO) proteins might play an important role in the posttranscriptional regulation of this symbiosis. Very recently, we demonstrated that the expression of *AGO5* is induced during the first three-hours of interaction with rhizobia. Likewise, we demonstrated that the down-regulation of *AGO5* in both common bean and soybean transgenic roots affects the expression of symbiosis-related genes, as well as the development of both infection threads and nodules. To clarify the role of AGO5 in the legume-rhizobia symbiosis, we edited the genome of transgenic roots in soybean using CRISPR Cas9. Transgenic roots Cas9-AGO5 were inoculated and we evaluated the nodule development. A 300bp deletion in the AGO5 gene was confirmed. Currently we are analyzing the phenotype of soybean transgenic roots expressing the Cas9-AGO5 construct.

112. Contribution of microRNAs miR482, miR2118 and miR1510 to the regulation of early stages of nodulation between *Phaseolus vulgaris* and *Rhizobium tropici*

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Legumes establish a symbiotic relationship with specific soil bacteria called rhizobia, resulting in the formation of specialized root organelles called nodules, where the rhizobia will differentiate into bacteroids and synthetize nitrogen compounds useable by the plant. The success of this interaction depends on the recognition of the specific partner and the exclusion of possible pathogenic bacteria as the rhizosphere is one of the richest microbial ecosystems on Earth.

Communication between the symbionts is necessary for the molecular and physiological changes that allow the initial stages of nodulation. Recent studies have demonstrated that recognition of molecular patterns that belong to rhizobia and other bacteria present in the rhizosphere, is in many cases necessary for successful symbiotic nitrogen fixation.

As part of its immune system, plants have a diverse family of proteins that function as cytoplasmic receptors to recognize strain-specific effectors. These proteins contain two domains: a nucleotide binding site and leucine rich repeats (NBS-LRRs). Their regulation by miRNAs, a group of small RNAs involved in post-transcriptional gene regulation, has gained relevance since the role of miRNAs during development, signal transduction and stress responses has been determined.

Three families of miRNAs have emerged as important regulators of NBS-LRRs: miR482, miR2118 and miR1510. Nevertheless, their participation during the establishment of symbiosis has not been studied. Thus, we are characterizing the accumulation of these miRNAs and confirming selected mRNA targets during the early stages of nodulation between *Rhizobium tropici* and *Phaseolus vulgaris*. We propose that regulation of immune responses is required during early stages of plant-bacteria interaction, especially for molecules such as NBS-LRR receptors, to allow *Rhizobium* to enter into the root and develop and adequate interaction.

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113. Endophytes non- rhizobiales isolated from nodules of *Mimosa pudica* with biotechnological potential

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The legumes have the ability to make symbiosis with different microorganisms, many nude bacteria are considered plant growth promoting bacteria (PGPB), due to their ability to stimulate the growth of plants, through direct and indirect mechanisms. There are several reports of non-rhizobial endophytic bacteria with the ability to stimulate plant growth (Zaheer et al., 2016), so in this work we evaluated the capacity of 10 isolated *Mimosa pudica* nodules, with analysis of the 16s gene were identified belonging to the family Enterobacteriaceae and results of the characterization of their ability to promote plant growth (production of indole acetic acid, phosphate solubilization, siderophore production, antagonism against phytopathogens) showed that they have several applicable characteristics to be PGPB, in addition 2 isolates identified as Enterobacter sp. have the presence of the *nif*H1 gene. The effect of the isolates on the growth of bean plants was evaluated, although all the isolates have characteristics of PGPB, only some had beneficial effects in the growth promotion of bean plants compared with the controls, likewise the type of interaction that exists between one of the isolated with the presence of the *nif*H1 gene with the roots of bean plants was studied, the ability to colonize nodules in the presence of a rhizobial bacterium (*Rhizobium etli*) was demonstrated however the ability to form nodules and fix nitrogen was not demonstrated.

Reference: Zaheer, A., Mirza, B., Bur, S., Mclean, J.E., Yasmin, S., Shah, T.M., Malik, K. y Mirza, M.S. 2016. Association of plant growth-promoting *Serratia* spp. with the root nodules of chickpea. Research in Microbiologoy, doi: 10.1016/j.resmic.2016.04.001.

114. Identification and cloning of a defensin from *Bixa orellana* L.

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Bixa orellana L, is a large, quick growing tree, native from tropical America, and is cultivated in the Caribbean and Central and South America. The pigment bixin is an apocarotenoid found in the aril coating the seeds and can be prepared in a mixture of compounds called annatto. The annatto paste, has different industrial applications, as a dye for natural fibers or as coloring additive in food and cosmetic industries. Nowadays the economical importance of *B. orellana* is based on *bixin* production (Rivera-Madrid *et al*., 2016). In this work we present defensins as candidates to study new molecules from *B. orellana* transcriptome and their potential use as antimicrobial agents.

Defensins play important roles in plant defense against fungal pathogens. Plants as sessile organisms that are exposed to numerous biotic or abiotic stress factors, have evolved complex mechanisms to deal with microorganisms. Being studied as part of the innate immune response, defensins are conserved antimicrobial peptides, also present in vertebrates and invertebrates (Do *et al*., 2004). Plant defensins are mainly cationic and antifungal peptides with a structure assigned to the alpha-beta cysteine stabilized family (αβCS) of globular proteins, plant defensins contain from 45 to 55 amino acid residues and eight cysteines that form disulfide bridges (Van der Weerden *et al*., 2013). We have identified transcript sequences that code for the mature peptide similar to two defensin-like peptides from Medicago and Citrus genera, present in the *B. orellana* L. transcriptome. The specific primers for each defensin were used to obtain the cloned CDS of BoDeF1 and BoDEF2, respectively. The purpose of this work is to obtain the plasmid constructs to produce the two “achiote” defensins in a bacterial expression system.

115. Isolation and characterization of bacteria associated with native maize from acid soils

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The maize (*Zea mays*) is one of the most important crops in the world. In Mexico, the corn is a representative crop because of its economic, social and cultural importance, but it faces damages caused by the excessive use of agrochemicals such as nitrogen fertilizers that promote phosphorus removal. Phosphorus is one of the most important elements in the growth and development of plants. The plants interact with a wide variety of endophytic and rhizospheric bacteria which promote plant growth and behave as biological control agents against pests and diseases, representing an important source of biotechnological and sustainable alternative to agrochemicals. In this study we carried out the isolation and characterization of endophytic and rhizospheric bacteria associated with native (criollo) maize from acid soils in the state of Michoacán. Fifteen isolates were selected by their ability to solubilize phosphates, they were evaluated by direct contact, distance and bacterial volatile organic compounds (VOCs) tests in *Arabidopsis thaliana*, identifying isolates with plant growth promoting effect through an increase in primary root length, lateral roots number and an increase in foliage and root biomass. We evaluated differents Arabidopsis lines reports such as auxins responses (*DR5::uidA*), cell division (*CycB1::uidA*) and phosphate transporters (*ATPT1::uidA*) to understand the molecular mechanisms of plant growth in Arabidopsis. Besides, we evaluated the isolates with phosphate solubilizing capacity, obtaining percentages of solubilization in some isolates with values of 50-70%. Our results indicate that in some bacteria there are a great solubilizing potential and when these are in direct contact with the plant, as well as inoculated at a distance, they induce a response to stress due to phosphorus starvation. In addition, we found that some isolates produces volatile organic compounds (VOCs) with auxin activity promoting the mitotic activity.

116. Short day photoperiod and arbuscular mycorrhizal symbiosis induces rebaudioside A accumulation in *Stevia rebaudiana*.

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Stevia rebaudiana is an important economic crop and is unique to produce steviol glycosides (SGs). In general, stevioside is the predominant SGs accumulated in leaves, followed by rebaudioside A. SGs are about 300 times sweeter than sugar. Arbuscular mycorrhizal (AM) symbiosis is the most important interaction between plants and soil fungi, which improves nutrient uptake, and reduces susceptibility to several biotic and abiotic stress conditions. In addition, plants exposed at different photoperiods (short and long days) could be affected their physiological and biochemical responses. Little is known about the effect of different photoperiods on arbuscular mycorrhizal colonization, as well as on SGs accumulation in *S. rebaudiana*. Therefore, the objective of this study was to investigate the effect of short and long days, and AM symbiosis, on plant growth, chlorophyll fluorescence, and on SGs content in *S. rebaudiana*. Results indicate that short and long days photoperiod had no effect on total mycorrhizal colonization, however, at short day conditions the number of arbuscules per length of infection units significantly decreased compared to long day treatment. Additionally, colonized plants exposed to short and long days contributed to increase height of shoots, as well as fresh weight of leaves and roots to non-colonized plants. Interestingly, the photosynthetic activity was higher in colonized plants under long day condition than non-colonized plants, whereas, short day plants presented no significant differences on photosynthetic activity, regardless their symbiotic status. Finally, colonized plants grown under short day condition presented a higher content of rebaudioside A than non-colonized plant. This work was funded by CONACYT (480787 and 702975) and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN (20180427, 20196531 and 20195064).

117. Exogenous phosphate regulates the arbuscular mycorrhizal symbiosis establishment and photosynthetic performance in *Stevia rebaudiana*.

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Stevia rebaudiana is an important economic crop to produce steviol glycosides (SGs), which are about 300 times sweeter than sugar. Arbuscular mycorrhizal (AM) symbiosis is a biotechnological strategy used in agriculture to optimize nutrient acquisition, such as nitrogen and phosphorus (P), as well as a reduce susceptibility to several biotic and abiotic stress conditions. P concentration determine AM symbiosis establishment, and can influences positively or negatively on photosynthetic performance. However, while P fertilization complement the P deficiency, it reduces AM symbiosis with the added benefits. In the present work, four exogenous phosphate concentrations (20, 200, 500 and 1000 µM) were applied in *S. rebaudiana* plants in order to determine the effect on AM symbiosis establishment by *Rhizophagus irregularis*, and the relation between AM symbiosis and the exogenous phosphate on photosynthetic performance. We observed that 20 and 200 µM phosphate induced high percentage of colonization, as well as arbuscules development. However, the applied of high phosphate 500 and 1000 µM drastically reduced AM symbiosis. *Arum*-type colonization was identified by confocal microscopy analysis. In addition, colonized plants at 20 and 200 µM phosphate contributed to increase the P uptake, Mg content and photosynthetic pigments (chlorophylls and carotenoid), compared with the corresponding non-colonized *S. rebaudiana* plants. Iinterestingly, we observed that AM symbiosis compensated the stressful condition by phosphate deficit that 20 and 200 µM caused to non-colonized plants, restoring the photosynthetically activity. Whereas plants fertilized with high phosphate concentrations (500 and 1000 µM), presented no significantly differences on photosynthetic performance. This work was funded by CONACYT (480787) and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN (20180427, 20181785 and 20195064), and DGAPA IN-207814.

118. Selection and evaluation of endophytic bacteria of plants of the *Poaceae* family of the Reserva la Uba, Guasave, Sinaloa with biotechnological potential and antagonistic to *Rhizoctonia zeae*

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In Sinaloa, corn is one of the most profitable grain crops, which has led to its monoculture, which has led to the appearance and increase of problems caused by fungal diseases, as is the case of *Rhizoctonia zeae*, that last years the incidence in the maize has increased, causing stems rot and roots. To fight diseases of this type the biological control is used, however, the effectiveness has not the expected because the microorganisms used are exotic bacteria. From this is derived from the importance to use native organisms of the region for the biological control, such is the case of endophytic bacteria isolated the giant reed and maize, that which the by sharing the same ecological niche with the pathogen are a potential to combat the rhizoctoniasis. It was used the leaf, stem and root of the plant giant reed and maize to perform the isolation, eleven γ-hemolytic strains were selected with antagonistic capacity to *R. zeae* with percentages of inhibition that ranged between 21 and 50%. Likewise, its made a characterization for to know its ability to promote growth, having as result that a strain producing chitinase, six strains produce glucanases and proteases, eight strains produce IAA in a range of 0.013 and 1.60 µg / mL, six strains produce siderophores and six present the ability to solubilize phosphate. To complement the characterization of the bacterium, it was used the model of *G. mellonella* to evaluate the virulence that they presented, resulting in that three strains were virulent. The Molecular identification was performed of the strains: *Rhizobium pakistanense, Staphylococcus warneri, Bacillus aryabhattai, Staphylococcus saccharolyticus, Acinetobacter radioresistens, Bacillus velezensis, Pseudomonas guariconensi, Pseudomonas plecoglossicida, Pseudomonas aeruginosa.* Considering the above, it was concluded that the strains isolated from giant reed and maize have the potential to promote plant growth and biological control to *R. zeae.*

NATURAL VARIATIONS AND GENOME EVOLUTION

119. The plastome of Hechtia pringlei (Bromeliaceae: Hechtioideae)

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Chloroplast are the organelles responsible for carrying out photosynthesis and other metabolic processes in eukaryotes. The chloroplast genomes or plastomes have been employed as a source of information for evolutionary studies as they provide information for phylogenetics analysis, even at high levels of taxonomy. In this work we present the first sequence of the plastome in Hechtia (Bromeliaceae: Hechtioideae), a genus endemic to Megamexico. The objective of this work is the characterization of the organization of the plastome in Hechtia pringlei B.L. Rob & Greenm, as well as its comparison to the closest species with a known plastome sequence, Tillandsia usneoides (L.) L. (Bromeliaceae: Tillandsioideae). The size of plastome of H. pringlei, the large single copy (LSC), short single copy (SSC) and two inverted repeated regions (IR) were characterized and compared to those in T. usneoides, as well as the number of genes, number of tRNAs a duplicated gene in the region IR. Both plastomes differ in size, number of tRNA and localization of genes like ycf-3 (hypothetical chloroplast reading frames).

120. The complexity of folate polyglutamylation in plants: ripening and ethylene modulate polyglutamylated profiles in climacteric fruits plus systematic analysis of the glutamyl tail-editing enzymes

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Folate derivatives exist in nature in a variety of polyglutamyl forms (Glun), the glutamyl tail is added to the folate molecule by folylpolyglutamate synthetase (FPGS) and removed by gamma-glutamyl hydrolase (GGH) isoforms in a number of compartments within the cell. Folate polyglutamylation affects the use of the folate cofactors and their transport in organisms impacting also their bioavailability as vitamins in mammals; however, little is known about its regulation in plants. We profiled the polyglutamylation extent of the main folate in climacteric fruits, 5-CH3-THF, and demonstrate that the profile is dynamic through ripening and it is affected by exogenous ethylene gassing being long Glun tails more susceptible to the treatment than fruits with short ones. In addition, we retrieved and compared deduced FPGS and GGH sequences from plants with known Glun profile and attempted to correlate their copy number, predicted localization, and primary sequence with the Glun profiles generated and gathered by this study. We present evidence of developmental, environmental and possible genetic factors impacting Glun extent in plants.

121. Genetic variability within three populations of *Marathrum rubrum* (Podostemaceae) suggests sexual propagation and colonization of its habitat

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Podostemaceae is the largest family of strictly aquatic angiosperms, including 48 genera and over 270 species. Unlike other aquatic plants, which reproduction is mainly asexual, relying dispersion on vegetative structures, in Podostemaceae the sexual reproduction seems to be the main process of colonization according to the high number of seeds per fruit. Some vegetative structures of *Marathrum rubrum*, such as root hairs and their association with Cyanobacteria, are clearly specialized in keeping the plant attached to the substrate, which usually are rocks under strong running water (rivers or water falls). These vegetative structures could be also involved in the propagation of the plant in order to colonize the rocks where they grow, suggesting the that a rock is only occupied by one individual. However, *M. rubrum* produces around 500 seeds per fruit and every single population produces thousands of fruits, indicating that sexual reproduction is a relevant factor in its propagation. Despite of this, the scope of the sexual reproduction in this species is still unknown due to the growth habit it exhibits and the apparently high degree of displacement of both vegetative propagules and seeds, leading the question if the colonization of the rocks to which the plant adheres is due to asexual propagation or sexual reproduction. In this study, we assessed the genetic variability by microsatellite analysis of three populations (i. e. three different points of the same river) of *M. rubrum* in Jalisco, Mexico. Three samples were taken from one rock and three rocks were randomly chosen from each location of the river. The results indicated that most of the samples are different individuals, including those from the same rock. Therefore, it is possible to propose that the populations of *M. rubrum* are produced by sexual reproduction and that this mechanism is the one that participates in the colonization of the rocks in which all individuals germinated.

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122. Genetic Variability in Different Species of Agaves Used in Distillery Using ISSR Markers

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The genetic variability between the different species of agaves and their agroecological association is a key element that will allow using tools to use biotechnological tools such as micropropagation to potentiate the development of the Agave-Tequila and Agave-Mezcal production chains.The aim of the present work was to select the ISSR markers (Simple Repeated Intergenic Sequences) capable of detecting polymorphisms in *Agave tequilana*, *Agave angustifolia*, *Agave potatorum* and *Agave karwinskii* species. A total of 26 primers were tested, which showed differential amplification patterns between the different species of agaves. Six primers were selected because they had a consistent and differential amplification pattern. Through an UPGMA analysis, a dendrogram was generated with a clear differentiation between species and grouping according to their taxonomic level. The results reported in the present work demonstrate for the first time an effective tool for the genetic differentiation between the species of *A. tequilana*, *A. angustifolia, A. potatorum and A. karwinskii*, which will allow continuing the study of genetic diversity at level of intraspecific populations.
Key words: molecular marker, genetic variability, ISSR.

PLANT RESPONSES TO THE ENVIRONMENT AND CLIMATE CHANGE

123. Participation of the PLETHORA transcription factors in the determinate growth of the primary root of *P. pringlei* (Cactaceae)

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The Root Apical Meristem (RAM), which is located at the root tip and produces new cells through strictly regulated division patterns to sustain root growth. In most plants the RAM is maintained for long periods and the root can grow indefinitely. The RAM of the primary root of Cactaceae is exhausted shortly after germination and the root stops growing. This is known as primary root determinate growth, and it has been proposed as an adaptation to arid/semiarid environments. Cell proliferation in the RAM is regulated by several pathways, being the PLETHORA (PLT) transcription factors the most important for RAM specification and maintenance. In *Arabidopsis* roots, PLT proteins display gradient distribution, their activity is dosage-dependent and defines root zonation. The *Arabidopsis* genome encodes 6 *PLT* genes; *A. thaliana* loss-of-function double mutants of *PLT* genes exhibit root determinate growth. To address the relevance of PLT in root determinate growth in Cactaceae, we identified the *Pachycereus pringlei PLT* from transcriptome data and evaluated their expression levels across developmental stages. To explore the evolution of *PLT* genes in land plants, we extracted 1,216 PLT-like sequences from 62 species and used them to construct a phylogeny. We found one *P. pringlei* ortholog for each *Arabidopsis PLT* gene. Contrary to *Arabidopsis, P. pringlei PLTs* are expressed even after RAM exhaustion, *i.e.*, when all root apex cells are differentiated, suggesting additional functions of these proteins besides RAM maintenance.

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124. Understanding of the transcriptional mechanisms that regulate the MEP pathway

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To understand the transcriptional regulation of methyl-D-erythritol 4-phosphate (MEP) pathway genes, tools like reporter gene systems have been developed. One of these is firefly luciferase (LUC) whose properties make it a very good reporter for *in vivo* quantification and image of transgene promoter activity since it is a non-invasive plant analysis procedure that allows the observation of luciferase activity during a time-lapse in the same individual. In this study, the gene expression of the MEP pathway was analysed cloning promoters of deoxyxylulose phosphate reductoisomerase (DXR), methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS), 4-Hydroxy-3-methylbut-2-enyl-diphosphate synthase (HDS) and 4-Hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) upstream of the firefly luciferase gene (LUC) in the pBGWL7 using gateway technology to produce transcriptional fusions. *DXR::LUC, MCT::LUC, CMK::LUC, MDS::LUC, HDS::LUC, HDR::LUC* promoter:: reporter constructs were introduced into *Arabidopsis thaliana* for selection of resistance to methionine sulfoximine (MSO) lines. We will use an in vivo assay of firefly luciferase activity to explore promoter strength, tissue specificity, and temporal regulation during the development of the MEP pathway genes simultaneously. The advances in this work will be shown.

125. Two epiphytic orchid species from Northeastern Mexico differentially express water stress response genes

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Water is an essential factor for the plant metabolism; in the epiphytic environment, the roots of plants are not contacted with the soil and water is obtained directly from the atmosphere. Hence epiphytes are prone to drought. In the tropical mountain cloud forests, epiphytes experiment a deficiency of water resource because it occurs a short dry period, that may cause severe water stress deriving in cellular and molecular changes. Some vascular plants limited water period places; they own adaptation environmental stress through crassulacean acid metabolism (CAM). Phosphoenolpyruvate carboxylase catalyzes the initial fixation of atmospheric CO_2 into oxaloacetate and subsequently into malate. We studied the physiological and molecular responses of the orchids *Stanhopeatigrina* and *Prosthecheacochleata* under drought and rewatering conditions. The plants were maintained under three levels of light: 20%, 50% and 70% of total daily radiation, during 35 days of water stress and eight days of water. We analyzed differential expression of three response genes to water stress; encode for CAM metabolism and a signaling process. Using genes expressed study species under water stress, described in NCBI. Using qRT-PCR starting cDNA samples obtained, a useful mRNA expression pattern is expected during drought treatment. *Stanhopeatigrina* was the most affected because it exhibited strong photoinhibition and reduction of both electron transport rate and nocturnal acidity under drought and high radiation. However, this species maintained relatively high relative water content (RWC) values and underwent osmotic adjustment during the drought period and recovered photosynthetic variables during the watered period. *Prosthecheacochleata* maintained similar to water and photosynthetic responses to light conditions during the drought period and was more tolerant than *S. tigrina*.

126. Genetical and biochemical characterization of two maize varieties under abiotic stress conditions

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Abiotic stress is a major factor in reducing growth, development and yield of plants. In response to abiotic environmental stresses, plants have many adaptive strategies include changes in physiological, biochemical and molecular processes. In this project, seeds of two maize (*Zea mays*) cultivars (MPR017-UG and MGA21- UG) were germinated and grown for three weeks under greenhouse conditions. Drought stress consisted in withholding water to ten plants for seven days and then re-watered to allow a fully recovery. Control plants were kept under well-watered conditions. Salinity stress condition was developed by a daily treatment with equal volume of different concentrations (from 0 to 300 mM) of NaCl, increasing gradually 50 mM per day until final concentrations in 10 days. To evaluate plant response to abiotic stress, the osmolites accumulation (proline, glucose, fructose and sucrose), starch, antioxidant enzyme activity (SOD, CAT, LOX, APX) and gene expression (*Zmsod2, Zmsod9, Zmmdhar2, Zmgr1, Zmapx*) were determined. A higher amount of soluble sugars and a lower amount of starch were found under stress in both varieties. Soluble sugars concentration increased (from 1.11 to 1.81 times) in shoot of plants subjected to drought stress, whereas starch content decreased significantly (from 67% to 79%). The free proline level also increased (from 1.08 to 1.65 times) in response to drought and from 2.54 to 6.2 times under salt condition in both varieties. It seems that in maize, proline is involved in minimizing the damage caused by dehydration and salinity. Antioxidant enzyme activity (LOX and APX) increased in both genotypes, however MGA21-UG induced CAT and MPR017-UG SOD activity. Similar results were obtained in qRT-PCR analysis. Therefore, it can be concluded that the stress tolerance mechanisms are different in seedling stage of both maize genotypes.

127. Changes in protein ubiquitination in maize seedlings (*Zea mays* L.) during the root hydrotropic response.

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High temperatures, flooding and drought are very highly stressful conditions for plants. Drought is one of the major causes of crop yield reduction, which causes substantial losses to the economy by increasing poverty and malnutrition. Roots are the first organ to detect this stress and use tropic responses to explore and direct growth to favorable conditions of water and nutrients. Differential root growth in response to a soil moisture gradient is defined as hydrotropism. Maize roots with a robust hydrotropic response (> 40^{o} of curvature) are able to maintain root biomass production as well as grains in drought. Under this condition, it is essential to ensure the stability of cell membranes, as well as macromolecules inside the cell. Proteins are especially sensitive to denaturation and cells have a system to degrade them using the proteasome and enzymes responsible for protein ubiquitination (Ub). In maize seedlings with a robust hydrotropic response, the Ub-proteasome system differs from those roots with a weak hydrotropic response (>39-10^{o} of curvature), which might be crucial for conferring drought tolerance. The subsequent analysis of the genes involved in this response, could provide alternatives to improve resistance in maize and another crops. Taking into account the relationship of Ub-proteasome with drought tolerance in the present work, we will study the changes in ubiquitination of proteins during the hydrotropic response of lines provided by Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMyT) with a robust and weak hydrotropic response.

128. Effects of a ventilation system on *Vanilla planifolia* Jacks *in vitro* plants

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In vitro plant tissue culture in sealed and ventilated sterile containers enables vanilla plants to grow under aseptic conditions to avoid contamination by fungi and bacteria. It has been suggested that controlling the physical vessel environments of tissue culture explants can improve the morphological characteristics of plantlets and their acclimatization to *ex vitro* conditions. The practice of using sealed containers increases the concentration of gases such as ethylene and carbon dioxide, which promotes morphological and physiological changes of the plants. These alterations can cause a low survival rate during *ex vitro* adaptation. Accordingly, the use of ventilated containers may facilitate the reduction of water vapor in the containers and increases of gaseous exchange, which improve the vigor of plants and their survival rate. Thus, in this study we assess shoot production and the development of *Vanilla planifolia* plants grown *in vitro* using two types of containers: closed (sealed) and ventilated with filter covers. The production of shoots was done in MS medium with sucrose and BAP, whereas, the plant developed in MS medium with sucrose. Plants monitored for 60 days, at 0, 15, 30, 45 and 60 days were the following parameters were evaluated: 1) number of leaves, 2) number of roots and shoots, 3) height reached, 4) number of stomata and their quality, 5) quality of seedlings (no chlorosis, or necrotic areas). Plants grown in ventilated containers exhibited increased shoot and root number compared in contrast to vanilla plants cultivated in sealed vessels. The height and leaf number were enhanced in vanilla plants developed in the ventilated container.

129. Characterization of flaxseed mucilage for its use in a natural sunscreen

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The sun is a source of ultraviolet radiation that reaches the earth's surface and can cause skin lesions associated with cell destruction and damage to tissues such as swelling and fluid loss, among others. To avoid these adverse effects, a special type of cosmetic products, the sunscreens, has been developed. Lately, some alternatives have been sought in the development of these products due to the increase in ultraviolet radiation that affects the earth's surface as a result of the thinning of the ozone layer. A motivating alternative in photoprotection are the beneficial properties of some seeds for people, including flaxseed. One of the most known and interesting components of flaxseed is its mucilage due to its properties and functions. Flaxseed mucilage has the property of absorbing ultraviolet radiation. We took advantage of this property to elaborate a sunscreen with an emulsion of natural lipids and water. The sun protection factor of this sunscreen was determined *in vitro* by means of spectrophotometric measurements to evaluate its photoprotective activity. The sun protection factor obtained for this photoprotective cream was 8. This sun protection factor can be increased using higher concentrations of flaxseed mucilage in the formulation. This formulation must be optimized to adapt to different skin types.
This work was founded by Universidad de Guanajuato CCS and DCSI.

130. Phylogeographic analysis of the determinate growth of the primary root in Cactoideae (Cactaceae) species

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The Cactaceae family includes ~1,600 species, most of them well adapted to arid or semi-arid environments. We previously reported that Cactaceae species show determinate growth of the primary root as a consequence of root apical meristem (RAM) exhaustion (Dubrovsky 1997; Shishkova *et al.,* 2013). Upon RAM exhaustion, all cells in the root apex differentiate and root growth ceases. It was suggested that the determinate growth in Cactaceae represents an evolutionary adaptation, allowing rapid seedling establishment in deserts. We surveyed the incidence of determinate growth of the primary root in the Cactoideae subfamily, which includes ~1,200 species. Primary root RAM exhaustion was evidenced by DIC microscopy of cleared roots. A maximum likelihood tree using 3 plastidic and 1 nuclear marker of Cactaceae species was constructed. The ML tree was used to map the root growth type and natural distribution for each species, to infer the relationship between these traits. 119 species, from 46 genera analyzed in this and previous work, had determinate growth of the primary root; *i.e,* underwent RAM exhaustion soon after germination. Remarkably, these species inhabit arid or semi-arid environments, strengthening the hypothesis that the determinate growth of the primary root in Cactoideae species is concomitant to water depleted habitats.
Acknowledgement: This work was partially funded by UNAM-DGAPA-PAPIIT (IN201318, IN200818) and CONACyT (240055) grants.

REGULATION OF GENE EXPRESSION

131. Exploring the function of the transcriptional factor ABI4 in *Marchantia polymorpha,* and its role on the evolution the land plants

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Land plants evolved from Charophyceae algae, from which they inherited the biological, biochemical, physiological and cellular attributes, thus facilitating the colonization of the terrestrial environment. During this process, the first three divergent lines of terrestrial plants (liver, moss and bryophytes) were generated, they lack vascular tissues and green roots, but they have morphological and physiological adaptations, which allowed them terrestrialization. One of these ancestral organisms is *Marchantia polymorpha*, which is a good model for studying ancestral signaling pathways, which were important during colonization and adaptation to the terrestrial environment. In this project, the perception and signaling by sugars and the ABA hormone were evaluated during Marchantia polymorpha development, through the analysis of the function of the transcription factor (TF) ABSCISIC ACID INSENSITIVE 4 (ABI4), which participates in the signaling of different processes. The ABI4 is very conserved in plants, and having found the possible ortholog in *Marchantia polymorpha*, its evaluation could give us relevant information about its function and evolution. Through analysis of the promoter, CRISPR-Cas mutants and overexpression plants, we have found that ABI4 may be involved in the asynchronous division and twisting thallus and therefore in the activity at meristematic sites; likewise, the possible preserved function of sugar signaling. This could suggest that gemma similar to seeds are structures subject to environmental regulations where ABI4 can also participate, so we believe that this protein could have a relevant function in signaling responses to environmental signals.

132. 'RIA3', the inducible region of *CrGPDH3* promoter of the green microalga *Chlamydomonas reinhardtii*

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The green microalga *Chlamydomonas reinhardtii* is a photosynthetic organism used as a model for studies in biology, biochemistry and physiology for more than 60 years. There are several tools for the genetic manipulation of this microalga, and its nuclear genome sequence is publicly available. *C. reinhardtii* has a great potential as a bioreactor for the production of recombinant proteins of biotechnological interest, it is relatively easy to cultivate, it shows a suitable accumulation of biomass and proteins, and it is a GRAS organism (Generally Regarded As Safe) by the FDA in the United States. However, protein yields have not been satisfactory to consider *C. reinhardtii* as a biotechnological platform of commercial interest for recombinant proteins. A possible solution is the use of promoters that drive high levels of gene expression. In *C. reinhardtii* constitutive promoters have been more studied than the inducible ones. Recently, our research group identified and characterized two nuclear genes, *CrGPDH2* and *CrGPDH3*, which code for glycerol 3-phosphate dehydrogenase (GPDH) enzymes. These genes are inducible by NaCl at low concentrations (from 5 mM) and at very short times (from 5 to 30 minutes) (Casais-Molina et al. 2016). More recently, we obtained a chimeric promoter (*RIA3/PromC*) comprising the inducibility region (RIA3) linked to the minimum promoter (*PromC*) of *CrGPDH3*. This chimeric promoter drived the inducible expression of *GUSPlus* reporter gene, and a significant increase in GUSPlus activity was observed in the presence of NaCl and KCl as inducers (Beltran-Aguilar et al. 2019). In this project, we are evaluating the ability of the RIA3 region of the *CrGPDH3* promoter, to confer inducibility to the strong constitutive promoter *HSP70A/RBCS2*, and we will evaluate its response to different inducers such as NaCl, KCl and sucrose.

133. AUXIN RESPONSE FACTOR (ARF) regulation by small RNAs in maize callus induction and plant regeneration

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AUXIN RESPONSE FACTORs (ARFs) belong to a major transcription factor family involved in the plant responses to auxins. These transcription factors play important roles during plant development and organ formation. Several ARFs are subject to tight expression control by small RNAs (sRNAs). Here we explored sRNA-mediated ARF regulation in maize somatic embryogenesis and plant regeneration. Particularly, microRNA (miRNA)- regulated ARFs and maize ARF3 transcripts targeted by trans-acting RNAs (tasiRNAs) were significantly enriched in embryogenic callus as compared to non-embryogenic tissues and showed distinct patterns during plant regeneration. Hence we performed closer inspection on miRNAs, tasiRNAs and ARFs during the dedifferentiation process and subsequent callus subcultures. Our results indicate dynamic ARF regulation in somatic embryogenesis induction and *in vitro* plant regeneration, supported by auxin gradients and signaling only in the embryogenic callus.

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134. Insights into the proteomic and metabolic analysis in response to high glucose in the non-vascular plant *Physcomitrella patens*

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Both microorganisms and multicellular organisms coordinate their metabolic activity concurring to changes in nutrient availability. Sugar levels in plants are fine-tuned according to the plant development stage and environmental factors through different signaling pathways impacting in their growth and development. Particularly, glucose signaling has been widely studied in the vascular plant *Arabidopsis thaliana*, but it has remained largely unexplored in non-vascular plants, such as *Physcomitrella patens*. We explored the dynamic changes in metabolism and proteins population after glucose exposure of protonemal tissues. We applied an ionomic-fingerprint analysis (DIESI-MS), carbohydrates and chlorophylls quantification, Fv/Fm determination and label-free untargeted proteomics to investigate *P. patens* response to high glucose treatment (300mM). The Biological process, Cellular component and Molecular function classifications of the differentially expressed proteins were determined. The results of these analyses showed that glucose feeding causes specific changes in Physmomitrella ionomic-fingerprint, carbohydrate contents and proteins accumulation, which is clearly different from those osmotically induced responses. These analyses unravel evolutionarily shared and differential responses between vascular and non-vascular plants. We thank INSTITUTO POLITÉCNICO NACIONAL, SECRETARIA DE INVESTIGACION Y POSGRADO SIP, COFAA and CONACyT for financial support.

135. *ABA Insensitive 4* (*ABI4*) is a target of Leafy cotyledon 2 (LEC2) during seed development, germination and silencing during vegetative tissue.

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The transcriptional factor (TF) ABI4 plays diverse roles in plant development and environmental responses (i.e sugars and ABA perception, lipid mobilization, lateral root formation, salt tolerance and mitochondrial retrograde signaling). *ABI4* is predominantly expressed in the embryo during seed development and during early seedling establishment. Analyzed how this central factor is regulated is essential to understand its function.
LAFL network including the LEC1, ABI3, FUS3, and LEC2 TF form a complex regulatory network essential to ensure the correct seed developmental process, including embryo morphogenesis, protein storage accumulation, chlorophyll degradation, and desiccation. As ABI4 shares spatial and temporal expression with the LAFL and some of the phenotypes caused by mutations of these genes are similar, we analyzed the possible regulatory role of the LAFL over the expression of *ABI4*.
We confirm that the LAFL factors regulate *ABI4* expression during seed development. In particular we observed that LEC2 is an essential positive regulator of ABI4 expression during embryogenesis and early seedling development and its absence (*lec2*) results in an ABA and sugar insensitivity phenotypes.
We demonstrate that the positive regulation of *ABI4* by LEC2 occurs at the transcriptional level and is mediated through the RY2 *cis*-element present in the *ABI4* promoter. Interestingly, we also demonstrated that RY2 is also the binding site of a yet-unknown repressor that is essential to eliminate *ABI4* expression in other tissues and developmental stages of the plants. Accordingly, mutation on the RY2 element results in absence of *ABI4* accumulation in the embryo and early plant development and in an ectopically expression in other tissues and developmental stages of the plant. Taken this data we propose that *ABI4* expression is under both positive and negative regulation.

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136. Analysis of the *Physcomitrella patens mettl3* mutant on development and stress response

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N6-metiladenosine (m6A) is an epitranscriptomic mark important for correct gene regulation processes. It participates in different aspects of RNA metabolism, as biogenesis, translation, stability, degradation and more. Impacting in processes like embryogenesis and tolerance to stress, like saline stress. The machinery that regulates m6A is composed by three proteins groups, the erasers, readers and writers. In mammals, Methyltransferase like 3 (METTL3) is a member of the writers group, which contains the methyl transferase activity *per se*. METTL3 is highly conserved among many eukaryotes such as yeast, plants and animals and its mutation causes a deleterious phenotype in some organisms. In this project, we aim to study the *Physcomitrella patens* METTL3 homologue (PpMETTL3) since this moss represents an interesting model for developmental, desiccation and evolutionary analyses. We are currently characterizing the phenotype of a *Ppmettl3* mutant (SM1) during its development and its response to stress conditions. We found that the mutation is not deleterious under vegetative reproduction. However, we observed a curly growth of the SM1 protonema, compared with the wild type which grows in a straight manner, as the lack of a normal growth against gravity direction. We hypothesize that such phenotype is due to an auxin metabolism deregulation given that, it controls cell differentiation and gravity response. We are also evaluating a set of auxin-regulated genes known to be involved in development and differentiation that could be regulated by PpMETTL3 causing the phenotype observed in the assays in SM1. In regard to the stress response we evaluated the gen *Pplea3,* which is positively regulated under salt and ABA treatment and we noticed a deregulation of this transcript on the mutant indicating that it may be regulated by PpMETTL3. These results reveal the importance of *Ppmettl3* in different biological processes, suggesting a role for mRNA methylation in moss.

137. Transcriptional profiling from Arabidopsis *mpk6* mutant by RNA-seq[1]

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The mitogen activated protein kinase (MAPK) cascades are a central signal transduction platform ubiquitous in the eukaryotes. A cascade is three-tiered, transducing input information through a stepwise series of phosphorylation events, leading to an appropriate output response having high fidelity. They include enzymes MPKKK, MPKK and MPK, which are activated sequentially by phosphorylation, playing a role in responses of abiotic and biotic stresses, as well as, in regulation of plant growth and development. In the Arabidopsis genome 20 MPKs enzymes are encoded, but due their functional redundancy, only few of them have been characterized. From these, MPK3 and MPK6 play a redundant role in the control of many aspects of plant development like flowering, embryogenesis, and stomatal patterning, besides both regulate growth and hormone signaling. Non-redundant functionality has also been demonstrated among them, since mutation of the Arabidopsis MPK6 gene results in altered root architecture and seed development that are independent of MPK3 function. Using RNA-Seq, we compared the transcriptomes of wild-type and *mpk3* and *mpk6* mutants, from root tissue of 5 days old seedlings. So, 2769 genes were differentially expressed between all genotypes with a False Discovery Rate adjusted at $P \leq 0.05$, whose fold change expression was higher than 1.5; the subsequent analysis of these genes showed that the changes in the expression of 2217 of these genes was completely independent of MPK3. Differentially expressed genes were enriched for generation of precursor of metabolites and energy, positive regulation of gene expression and, negative regulation of defense responses. For further analyzes, we only focus on those genes that correspond to transcriptional factors as they are possible phosphorylation targets of MPK6, finding a gene enrichment in the categories: regulation of biological processes, response to hormone stimulus and root system development. Actually, some of those genes are being molecular and functionally characterized.

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138. Computational identification of miRNAs and their targets in *Phaseolus vulgaris*

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MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression through a sequence-specific recognition of their targets, leading to degradation or inhibition of translation. In the case of plant miRNAs, they have been involved in a multitude of biological processes, from developmental processes to environmental stress responses. Massive sequencing by RNA-seq is becoming a widely used technique to discover plant miRNAs. However, if costs are taken into account, bioinformatics prediction is a valuable tool for miRNA discovery in plants. Among the tools available, ShortStack is the best for a comprehensive *de novo* annotation and quantification of miRNAs. Therefore, ShortStack was used in this study to predict miRNAs of common bean (*Phaseolus vulgaris)*, one of the most important legumes in the word. Such predictions were possible thanks to the high conservation of plant miRNAs, enabling the identification of known and novel miRNAs in *P. vulgaris* by homology analysis. In that sense, 30 miRNA sequences of *P. vulgaris* were identified, belonging to 20 miRNA families. If compared to previous studies, 16 of the 30 miRNAs were newly discovered, deriving from previously unidentified loci. In addition, categorization of potential target genes suggests that most of them are predicted to be involved in plant development, particularly in organ morphogenesis of floral tissues. Finally, determination of interaction networks allowed to identify the most important nodes among the potential target genes. Therefore, our work provides valuable information for further research regarding the biological functions of miRNAs in *P. vulgaris*.

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139. CRISPR-Cas9 as a tool to interrogate somatic embryogenesis in *Coffea canephora*

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has become a familiar term for researchers involved in biological sciences. In seven years CRISPR has made genome edition available for everyone due to its simple design, its versatility demonstrated in different species and relatively low cost. This amazing biotechnological tool is in continuous growth and development, but the already reported works have made us dream about finding the answers to the most important questions about functional genomics. In our case, the answers we are looking for are related with which genes are involved in the initial signal of cellular differentiation in the somatic embryogenesis process. Our study subject outstands as one of the most important crops and commodities worldwide, Coffee. Our approach consists of knock-out target genes of the *YUCCA* family involved in indole-3-acetic acid synthesis and auxin regulation genes of *GH3* family related to auxin conjugation process. We expect that the results obtained from this work contributes to the understanding of the basic science behind plant cell differentiation and also can generate biotechnological applications not only for Coffea but other wood species.

Keywords: CRISPR-Cas9, Cellular differentiation, Somatic embryogenesis, Coffea canephora

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140. A singular genetic trick in the course of evolution protects *Arabidopsis thaliana* from drought stress

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Gene duplication is a frequent phenomenon that contributes to the processes of adaptation and diversification of species. In plants, multigenic families, have expanded and diversifiedy from single ancestral genes throughout various duplication mechanisms. ATL gene family, a RING-H2 type ubiquitin-ligases (E3), is an example of gene family exclusive of plants. E3 enzymes are components of the ubiquitin-proteasome system, involved in the labeling of protein substrates for selective degradation. We study the promotor architecture of the duplC-ATL a sub-group of 6 duplicated *ATLs*. *AtATL78*, a member of duplC-ATLs, has been previously implicated in water deficit responses. A comparative structural analysis in Brassicaceae, indicates the occurrence of five TATA-box signatures within the promoter region of this group (up-TATA, TATA-0, TATA-1 TATA-2a and TATA-2b). Evolutionary history studies and analyses of gene expression of duplC-ATLs, suggest that up-TATA, TATA-0 and TATA-1, define an ancient zone that drives expression throughout reproductive tissues, and that TATA-2a and TATA-2b define a recent duplicated zone that drives expression throughout vegetative tissues. These analyses also revealed that TATA-2b was generated by a tandem duplication of a 30 nucleotides fragment, that only occurred within the promoter region of *ATL78* orthologs of lineage I of Brassicaceae. Remarkably, this duplicated TATA-box increases gene expression. A transcriptomic comparison of wild-type and *atatl78* mutant lines, strongly suggest that TATA-2b contributes to water deficit tolerance in *A. thaliana*.

141. The natural compound α-mangostin as an antineoplastic and antiviral agent in a cervical cancer model

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Introduction: The mangosteen three (*Garcinia mangostana L*.) exhibits a variety of pharmacological activities mainly attributed to its biological active compounds named xanthones. The xanthone α-mangostin (AM) has shown potent antineoplastic effects in different types of cancer. Interestingly, AM also exerts antiviral activity. Based on the above, we decided to evaluate AM antineoplastic/antiviral effects in human papilloma virus (HPV)-positive cervical cancer cells. The E6 and E7 oncogenes of HPV are carcinogenic and promote the expression of the oncogenic ether á-go-go-type-1 potassium channel (Eag1), while vimentin prevents the internalization of the virus.
Objective: To evaluate the antineoplastic effect of AM on cell proliferation, cell cycle distribution and Eag1, E6/E7 and vimentin gene expression in a panel of cervical cancer cell lines.
Methods: Cell proliferation and cell cycle distribution were evaluated by sulforhodamine-B assay and flow cytometry, respectively, in C33A (HPV-negative), HeLa (HPV-18+), SiHa (HPV-16+) and CaSki (HPV-16+ and HPV-18+); while gene expression was evaluated by qPCR in CaSki cell line since it has the highest number of HPV copies.
Results and discussion: AM inhibited the proliferation of all cervical cancer cell lines in a dose-dependent manner. The most sensitive cell lines were those with the highest number of HPV copies such as CaSki, followed by SiHa, HeLa and C33A cells, which may be related to AM antiviral effect. In addition, AM promoted cell cycle arrest in G1 phase in CaSki, and led to cell death in SiHa and Hela cells. Regarding AM gene expression effects, the phytochemical decreased the expression of E6, E7and Eag1 while increased that of vimentin.
Conclusion: AM could be an alternative for the adjuvant treatment and prevention of cervical cancer. Supported by CONACyT México, grant 256994 from RG.

142. Towards deciphering the genetic mechanisms of viability loss in *Cedrela odorata* L. seeds. I.

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Cedrela odorata L. is native from Mexico and one of the most economically important neotropical species for the international forest industry. However, its seeds cannot be stored over long periods due to rapid loss of vigor and viability, which represents a major problem for propagation. Due to the highly complex nature of seed architecture and processes implicated, knowledge about germination regulatory networks and mechanisms is still limited. These restraints are even bigger for non-model taxa such as *C. odorata*.
Therefore, the main goal of this study was to identify diferentially-expressed transcripts related to three conditions: quiescent, hydroprimed and germinated seeds to elucidate the main processes and genetic mechanisms involved in *C. odorata* germplasm viability loss during storage. We used seeds from an individual tree belonging to a progeny test located in the experimental station El Palmar, Veracruz, Mexico (INIFAP).
Three samples for each condition were used for RNA isolation with the Trizol® reagent and were sent to the Biotechnology Institute (UNAM) for sequencing. A *de novo* reference transcriptome was assembled with Trinity and transcripts quantification was carried out with Salmon. To compare differential expression between the study conditions we used DESeq2 and edgeR packages in R.
A total of 16609, 18251 and 17949 transcripts were identified for dry *vs* hydroprimed, dry *vs* germinated and hydroprimed *vs* germinated seeds, respectively. In the same order of comparisons, differential expression of transcripts ranged from 8404 to 10061, 11267 to 12351 and 6068 to 7755. These preliminary results indicate that such as it has been described in other biological systems, *C. odorata* seeds germination implies the increase of mRNA synthesis. Further analysis require the identification of the main pathways and genes involved in viability loss in this species germplasm.

143. Possible participation of GH3 genes during somatic embryogenesis of *Coffea canephora*

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Cellular differentiation (CD) is a complex process and surely does not depend on the action of a single molecule but a complex signaling network. Plant cells have the extraordinary property of regenerating a complete and functional organism, a clear example is somatic embryogenesis (SE), where the development of autonomous structures in response to exogenous and endogenous signals can start in the plant or *in vitro*, directly or indirectly and that requires extensive and coordinated reprogramming. The study of auxin homeostasis encompasses the understanding of four major processes (biosynthesis, transport, storage, and degradation), although significant progress has been made in the understanding of the molecular basis of transport, perception and response of auxin, the control of metabolism and its homeostasis through conjugation and degradation mechanisms remains less studied, it has been shown that the *Gretchen Hagen 3* (GH3) gene family inducible by auxin, conjugates indole-3-acetic acid (IAA) with Amino acids, in coffee, more than 90% of the endogenous content of AIA is inactive. The objective of this work is to obtain mutants with the purpose of study the importance of these genes during the embryogenic process. Using genome editing tools such as CRISPR will allow us to produce very precise mutations of genes that we hypothesize could be involved in the ES and thus explore its function.

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144. Transcriptomic response to glucose stimuli on the non-vascular plant *Physcomitrella patens*

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Sugars and particularly glucose have a dual role in vascular plants, that is, in addition to providing the energy and carbon skeletons necessary for the primary metabolism of the plant, it also acts as a signal molecule regulating its development, metabolism and gene expression at both transcriptional and posttranscriptional levels. In spite of the importance on this topic, most of the research in this regard has been carried out mostly in *Arabidopsis thaliana*, and occasionally other vascular plants; but has remained unexplored in non-vascular plants. *Physcomitrella patens*, a non-vascular plant from the bryophyte family, was used to get insights into the transcriptomic response to glucose stimuli (300mM of glucose). RNAseq analyses originated a list of 32,054 differentially expressed genes, 20,490 up-regulated and 11,564 down-regulated. Then these genes were further classified to determine the enriched Biological process, Cellular component, and Molecular function categories. The results of these and other bioinformatic analyses will be presented.

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145. Analysis of the expression pattern of genes regulating the synthesis and transport of auxins in somatic embryos of *Capsicum chinense* Jacq.

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Capsicum chinense Jaqc. (Habanero pepper), is a domesticated specie of *Capsicum* genus, popular for its pungency and highly demanded by industries because of its general chemical composition. This specie is considered highly recalcitrant to the somatic embryogenesis (SE) process, that is evidenced in the large number of deformed somatic embryos and the low percentages of germination obtained by current protocols. These facts limit the genetic improvement and metabolic engineering of this crop. Although this phenomenon has not been fully elucidated, several studies at the molecular level have revealed that the process is governed by an intricate genetic network, in which genes are regulated in response to hormonal signals, highlighting the role of auxins during the process. It has been demonstrated previously that the distribution of the auxin IAA in somatic embryos differs from that observed in zygotes and the expression patterns of some crucial transcription factors (TF) are altered. The aim of this study is to analyze the expression of *BABY BOOM (BBM)* and *LEAFY COTYLEDON (LEC1)*, TFs involved in the regulation of auxin synthesis genes, as well as that of the *PIN FORMED* genes *(PIN1, PIN3 and PIN7)* which code for transporters of this phytohormone. This will contribute to elucidate their implication in the establishment of auxin homeostasis in zygotic and somatic embryos and their possible relationship with malformations of somatic embryos in *Capsicum chinense* Jaqc.

This research was financed by the CONACYT grant to NSB (6036300001) and by a scholarship #728540 awarded to GPF. We thank GemBio Laboratory for the technical support

146. Transcriptome analysis of the induction of somatic embryogenesis in *Coffea canephora* and the participation of ARF and Aux/IAA genes

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The somatic embryogenesis (SE) process is a useful biotechnological tool to study the morpho-physiological, biochemical and molecular processes that take place during the development of *Coffea canephora*, in which plant growth regulators play a key role in cell differentiation. Among them, the indol-3-acetic acid (IAA), the most abundant natural auxin, has a fundamental role in the development processes of plants; so, understanding the molecular mechanism with which it acts would benefit the improvement of the crop. The IAA signaling is a fundamental aspect for carrying out the embryogenic transition in the somatic cells of plants, and some of the main components in this mechanism are the Auxin Response Factor (ARF) and Auxin/Indole-3-acetic acid (Aux/IAA) genes, which can activate or repress the expression of genes responsive to the auxins. The growing development of new generation sequencing technologies, as well as bioinformatics tools, has allowed us to broaden the scope of the SE study of different plant species and to identify the genes directly involved in it. In this work, transcriptomic analysis of the coffee genome and the identification of some genes differentially expressed during the different stages of the process of induction of the SE in *C. canephora* were carried out.

This project is supported by CONACyT-Fronteras 1515.

147. Characterizing alternative splicing events in *Phaseolus vulgaris* root nodule symbiotic - genes

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The vast diversification of proteins in eukaryotic cells has been related with multiple transcript isoforms from a single gene that result in alternative splicing (AS) of primary transcripts. Genome-wide analysis from publicly available RNA-seq libraries led to identify 85,570 AS events from *Phaseolus vulgaris* (common bean) in 72% of expressed genes and 134,316 AS events in 70% of expressed genes from *G. max* (soybean). These were categorized in seven AS event types with intron retention being the most abundant followed by alternative acceptor and alternative donor. Conservation of AS events in homologous genes between *P. vulgaris* and *G. max* was analyzed. The results revealed high conservation (18% - 37%), over *ca.* 15 – 20 MY divergence, and may point to the biological relevance of AS (Íñiguez et al. 2017).

This work predicted different AS events in *P. vulgaris* highly expressed genes in inoculated roots/nodules during the *Rhizobium tropici* N-fixing symbiosis among other tissues and conditions. We have experimentally demonstrated the AS events in *ca*. 10 genes expressed during the rhizobium symbiosis, through the amplification of the different exon-exon junctions predicted for the primary vs. the alternative transcripts. We experimentally demonstrated an AS event of exon skipping- and alternative acceptor-type in two genes annotated as Apoptosis-promoting RNA-binding protein (Phvul.001G141300) and Remorin (Phvul.001G135500), respectively. Results from expression analysis evidenced the high root/nodule expression of these genes and also a high proportion of the alternative transcript vs. the primary transcript, something that suggest a relevant role of AS in the control of symbiotic N-fixation in common bean. Current research from our group is aimed to decipher the possible biological significance of such AS events.

148. A tomato fruit coexpression network

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Tomato it is a crop of great commercial interest in the world, and has become a model plant for the study of fleshy fruits. In order to improve tomato fruit quality and yield, breeders search a better adaptation to climate change and enhanced tolerance to abiotic stress, while preserving fruit quality. Our aim is to build knowledge that will lay a solid scientific foundation for future biotechnological applications aimed at improving tomato fruit ripening under non-optimal conditions. The strategy we propose for the study of tomato fruit transcriptional regulation is the construction of an inferred co-expression tomato network, followed by its experimental validation. We used the Algorithm for the Reconstruction of Accurate Cellular Network (ARACNE) and RNA-Seq experimental data to create an inferred tomato fruit network, which is a powerful tool that will allow us to focus experiments, saving time, resources and effort. We have begun to analyze our network by identifying genes at the interphase between fruit ripening abiotic stress responses.building coexpression subnetworks, performing enrichment analyses and beginning experimental characterization of protein-DNA interactions.

149. Transcriptional function of maize E2Fa/b1;1 and E2Fc proteins

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The eukaryotic cell cycle is regulated mainly by cyclins and cyclin-dependent kinases (CDKs) that are functionally conserved among all eukaryotes. In mammal cells, the main targets for cyclin-CDK complexes during G1 are proteins of the "pocket" family: p107, p130 and the retinoblastoma protein (pRb), which negatively regulate the G1/S transition through their interaction with the E2F-DP family of transcription factors. In animal cells this family of E2F-DP factors performs an important role in the progression and control of the cell cycle by regulating the expression of sets of genes involved in the different stages of cell proliferation, among them the initiation of the S phase.
Studies developed in *Arabidopsis thaliana* have shown that the E2F-DP pathway is conserved in plants and members of the family have been classified in subgroups according to their role as activators or repressors of transcription. E2F-DP proteins have been identified in other plant species as well. We have identified 12 members of the E2F family in maize, that were aligned to the rice and *Arabidopsis* E2Fs to build a phylogenetic and thus propose a nomenclature for maize E2Fs. According with the resulting classification, we chose a maize E2F with putative activating characteristics (E2Fa/b1;1) and another with putative repressor activity (E2Fc) in order to investigate their function.

We found that E2Fa/b1;1 protein is really a transcriptional activator and could identify a 40 aminoacids sequence that seems to be the responsible of this trait. In contrast, E2Fc protein could not activate transcription, suggesting that it may be either a transcriptional repressor or a non-activating E2F.

150. Analysis of gene expression: *APETALA (AP), SHATERPROOF (SHP)* and *SPATULA (SPT)* in the zone of dehiscence of the fruit of *Bixa orellana* L

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Bixa orellana L. is a shrubt known as achiote in México, with almost spherical dense crown, that belongs to the order of Malvales and is the only genus in the Bixaceae family. Its fruit is an ovoid capsule formed by two valves, and a structure called dehiscence zone. In the fruits of *B. orellana* we find two types, one dehiscent (open) and another indehiscent (closed), this characteristic becomes noticeable when the plant is adult and the fruits are mature. When this zone separates, the seeds are exposed to the environment leaving them vulnerable to insects, water, wind, pH changes, in the same way facilitating the photooxidation of pigments.

The dehiscent morphotype are the contributors of the greatest amount of bixin compared to the indehiscent, so this work aims to analyze the expression of the *APETALA 2 (AP2), SHATTERPROOF (SHP)* and *SPATULA (SPT)* genes involved in dehiscence processes fruit in plants. Thus, a bioinformatic analysis was carried out. Expression study was evaluated in different stages of development fruit of two dehiscent and indehiscent variants of Bixa orellana L; expression was performed by PCR and real-time qRT-PCR. An *in situ* PCR methodology of the *AP2, SHP* and *SPT* genes will be established in order to know the tissue localization in the fruit.

Keywords: Dehiscence, Bixin, *APETALA 2*, *SHATTERPROOF* and *SPATULA*

151. YUCCA-mediated auxin IAA biosynthesis is required during of somatic embryogenic induction process in *Coffea canephora*

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Somatic embryogenesis (SE) is the process by which somatic cells, under induction conditions, generate competent cells; these cells undergo a series of morphological, biochemical and molecular changes to give rise to somatic embryos without the fusion of gametes. A key component in the induction process of SE is indole acetic acid (IAA). IAA is an auxin that is synthesized by enzymes encoded by YUCCAs (YUCs) genes of the flavin monooxygenase family. In this paper, we report that the biosynthesis of the IAA is central to the SE induction process. The quantitative genetic expression analysis qRT-PCR performed during the induction process of SE shows an increase in the expression of YUC1, which suggests that it has a crucial role in embryogenesis. Besides, we use a yucasin inhibitor to block the activity of YUCs enzymes, dramatically affecting the induction process of the and also the decrease in the signal of free IAA in the leaf explants. Our findings indicate that biosynthesis and the location of IAA plays an essential role during the induction process of the ES in C. canephora.

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152. Scientific topic: Regulation of gene expression *Cedrela odorata* L. seeds *de novo* transcriptome assembly and gene annotation

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Cedrela odorata L. (Spanish cedar) is a native species from Mexico and also one of the most economically important neotropical taxa. In general, tropical woody species are considerably less studied than other plants due to their long life cycles, genome size and lack of reference genomes. Therefore, the intricate mechanisms involved in the physiological and molecular processes governing the loss of germinative capacity of *C. odorata* L. seeds are not known yet. This impedes efficient management and conservation strategies, which has resulted in its classification as a vulnerable (IUCN) and protected species (NOM-059-SEMARNAT).

The goal of this study was to identify the main mechanisms and genetic factors involved in viability loss of *C. odorata* germplasm. As a first stage, with Trinity v.2.8.5 the *de novo* transcriptome assembly was performed and it includes over 95700 transcript models corresponding to 52490 genes. Then, we performed a preliminary functional annotation analysis of the seed transcriptome.

BLASTx and BLASTp (BLAST+ v.2.9.0) analyses relied on uniprot database. HMMER v.3.2.1 identified a total of 12 765 protein domains where up to 20% consisted in unknown function domains. ATP-related domains were among the most common protein families along with peptidases, coiled coil, glycosyl hydrolases and transferases. Results were compiled in a SQLite database for further gene ontology analysis.

The number of genes identified in our study is similar to that found in the only previous study concerning *C. odorata* transcriptome from botanized leaves, suggesting a similar basal gene expression in both tissues. The reference transcriptome generated in our study constitutes the first attempt to elucidate the main genes expressed in Spanish cedar seeds. It represents an important reference genomic information for further investigations concerning seed longevity, physiology and *ex situ* germplasm conservation, among others.

SECONDARY METABOLISM

153. Spatio-temporal variation of the antiviral components present in Caesalpinia yucatanensis leaves

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In previous studies related to the search for natural alternatives with antiviral property, it was identified that Caesalpinia yucatanensis extracts presented antiviral activity at the co-treatment level against influenza A (H1N1) virus. The antiviral evaluation of different collections made in Yucatan, Mexico by Cetina Montejo and May May (2013-2014), allowed to detect a variability in biological activity, therefore, in the present work, the main objective was to determine the concentration in C. yucatanensis leaf extracts collected during the dry and rainy season in three locations in the State of Yucatán, in order to determine the most favorable area and time for the collection of this species that allows the identification and quantification of active secondary metabolites . Samples are collected in the municipalities of Yaxcabá, Oxkutzcab and Sierra Papacal during the dry season (November to January) and the rainy season (May to July). The extracts obtained from the collection sites were evaluated by HPLC and CGM, then they were compared between them and the different epochs in which they were collected, qualitative and quantitative differences were found that were related to the environmental conditions, such as: humidity , temperature and soil type. In subsequent works, it is proposed to isolate and identify the secondary metabolite (s) responsible for the antiviral activity of C. yucatanensis and thus quantify its presence in the different collection sites to determine the best production conditions.

Key Words: antivirals,inluenza A, anti-hemagglutinin

154. Functional characterization of two dioxygenases putatively involved in bixin biosynthesis in annatto (*Bixa orellana* L.)

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Carotenoid dioxygenases (CCDs) are enzymes that have been implicated in the biosynthesis of a wide diversity of secondary metabolites with important economic value, including bixin. Bixin is the second most used pigment in the world's food industry worldwide, and its main source is the aril of achiote (*Bixa orellana* L.) seeds. A recent transcriptome analysis of *B. orellana* identified a new set of eight CCD members (*BoCCD4s* and *BoCCD1s*) potentially involved in bixin synthesis. We used several approaches in order to discriminate the best candidates CCDs genes. A RT-qPCR expression analysis was carried out in five developmental stages of two accessions of *B. orellana*. seeds with different bixin contents. The results showed that three *BoCCDs* (*BoCCD4-1*, *BoCCD4-3*, and *BoCCD1-1*) had an expression pattern consistent with bixin accumulation during seed development.

In a second selection round, the three CCD genes were analyzed by *in situ* RT-qPCR; in seeds tissue. Results indicated that *BoCCD4-3* and *BoCCD1-1* exhibited tissue-specific expressions in the seed aril.

To test whether the two selected CCDs had enzymatic activity, they were expressed in *Escherichia coli*; activity was determined by identifying their products in the crude extract using UHPLC-ESI-QTOF-MS/MS. The cleavage product (bixin aldehyde) was also analyzed by Fourier transform infrared (FTIR). The results indicated that both, BoCCD4-3 and BoCCD1-1, cleave lycopene *in vitro* at 5,6-5´,6´.

In conclusion, we found two candidate genes, BoCCD1-1, and BoCCD4-3, which appear to have the same in *vitro* lycopene cleavage activity, although they are located in different cell compartments. Our data suggest that both classes of enzymes may be responsible for carotenoids metabolism. The primary objective of this work was to characterize and identify the BoCCDs involved in the initial step of bixin biosynthesis, but the subsequent steps and the mechanisms by which these enzymes take part in bixin biosynthesis remain uncertain. Therefore, we propose three possible mechanisms by which BoCCD4-3 and BoCCD1-1 are involved in bixin biosynthesis.

155. Effect of salicylic acid on betalain accumulation in *Stenocereus queretaroensis* cell suspensions

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Salicylic acid (SA) belongs to a group of compounds, known as elicitors, which have been widely used to redirect secondary metabolism in order to obtain specialized products of interest. *Stenocereus queretaroensis*, a common cactus that grows in the center part of Mexico, produces edible fruits or pitayas that contain a high concentration of betalains. It has been demonstrated that these compounds are a suitable option to replace synthetic colorants in the food and pharmaceutical industries. Nevertheless, these pigments can only be obtained when cacti fruits are available, which happens for a limited time (April to June). The establishment of cell suspensions from the fruit's pulp represent a good alternative for the availability of betalains that can substitute synthetic colorants. In here, we tested the impact that exogenous applications of SA in increasing concentrations (50, 100 y 200 µM) could have on betalain accumulation. Polyphenols content and antioxidant capacity were determined in both the elicited and the original *S. queretaroensis* cell lines.

156. Phytochemical characterization and antioxidant potential of peduncles, leaves and stems of *Capsicum chinense* J.

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The habanero pepper (*Capsicum chinense* J.), is used in traditional medicine to treat diseases such as diabetes and cáncer, but there are few scientific studies that characterize phytochemical and pharmacologically their by-products, which are generally discarded (4.8 million plants and 108 million peduncles in 2015). Objective: Identify and quantify the majority compounds and determine the antioxidant potential of the peduncles, leaves and stems of *Capsicum chinense* J. var. Jaguar, grown on black and red soils from Yucatán. Methodology: obtaining flour by-products (oven drying); Obtaining extracts (ultrasound assisted extraction with MeOH 80 %); Determination of the antioxidant capacity of extracts (DPPH); Identification and quantification of polyphenols, vitamins, carotenoids and capsaicinoids (UPLC). Results: The peduncles and stems of the red soil had the highest percentage of inhibition (91.10 % ± 0.24 and 91.01 % ± 0.08). The peduncles of red soil showed the highest amount of chlorogenic, coumaric and cinnamic acids (97.24 ± 0.44, 17.73 ± 0.07, 28.60 ± 0.11 and 44.34 ± 1.70 mg / 100 g of dry weight (DW)). The peduncles of red and black soils exhibited the greatest amount of routine (44.34 ± 1.70 and 44.57 ± 0.95 mg / 100 g DW). The red soil leaves presented the highest amount of quercetin plus luteolin (64.59 ± 0.02 mg / 100 g DW) and vitamin C (16.71 ± 0.20 mg / 100 g DW). The black soil leaves exhibited the highest amount of catechin (52.34 ± 0.52 mg / 100 g DW) and vitamins A and E and β-carotene (0.84 ± 0.03, 25.58 ± 0.02 and 161.10 ± 8.20 mg / 100 g of DW). None of the samples showed gallic acid or kaempferol and in the stems of the both soils, only chlorogenic acid was detected (5.61 ± 1.27 and 2.01 ± 0.01 mg / 100 g DW). Conclusion: The peduncles, leaves and stems of *Capsicum chinense* J., var. Jaguar grown in black and red soils of Yucatán, due to its phytochemical content and antioxidant activity, could be used in the food and pharmaceutical industries.

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157. Acetogenin evolution during avocado embryo and leaf development

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Avocado fruit (*Persea americana*) is a highly nutritious food, and avocado leaves are used in folk medicine. Both tissues contain different bioactive metabolites such as aliphatic acetogenins (odd-carbon, fatty acid derivatives) that could be used to prevent microorganism growth or cancer cell development. This work quantifies eight different aliphatic acetogenins (Avocadenine, Avocadene, UPA, Persediene, Persenone C, Persenone A, Persin and Persenone B) in seed embryonic axis and cotyledon, and leaves during germination and leaf development. During seed germination, acetogenin contents change in both cotyledon and embryonic axis; moreover, both tissues showed different total acetogenin contents, cotyledons had 25 mg/g (dry weight, DW) while embryonic axis accumulated up to 10 mg/g (DW). In addition, acetogenin profiles were different between these seed tissues; cotyledon had Persenone A as predominant acetogenin while embryonic axis had more than 60% of the acetogenin pool composed by Avocadenine and Avocadene. During seedling development, we sampled very young leaves at different developmental stages. Acetogenin content in very young leaf declines as the sprout begins to grow during the first 14 days after leaf emergence. However, when we compared these contents with those from two-year-old tree leaves, acetogenin levels were higher in the tree leaves, suggesting that total contents could be reestablished later on during leaf development. Interestingly, in very young leaves, acetogenin profiles changed dramatically. Very young leaves from seedling had the complete eight acetogenin profile in similar quantities. Later in development, Avocadenine, Persenone A and Persenone C levels decline, leaving Persin as the majoritarian compound in tree leaves. This is the first time that a full acetogenin profile is characterized in leaf tissue. This work shows that aliphatic acetogenin metabolism differs between developmental stages and between different tissues in avocado.

158. Heterologous expression of the bixin biosynthetic pathway genes *BOCCD4-3, BOADLH3H1-1* AND *BOSABATH4* in *Escherichia coli*

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Plant-derived pigments are highly demanded by food, pharmaceutics and cosmetic industry because of innocuous and antioxidant properties. However, isolating pigments from plant sources faces many challenges such as long production times, lack of homogeneity in plantations, diseases and large cultivation areas. Thus, it is necessary alternative approaches for the synthesis of plant pigments. Production in a heterologous system such as *Escherichia coli* is a viable alternative because of their short life cycle, simple growth requirements and the independence of climatic conditions. *Bixa orellana* is a shrub native to the Brazilian Amazon and holds commercial importance because of the bixin apocarotenoid, the second most important natural pigment in world after saffron, which accumulates in the seeds. The transcriptome analysis in immature seeds of *Bixa orellana* revealed a putative new bixin biosynthesis pathway (Cardenas-Conejo *et al.,* 2015). We cloned *Boccd4-3*, *Boaldh3h1-1* and *Bosabath4* involved in the bixin biosynthesis pathway from cDNA of immature seeds of *B. orellana*. The cDNA sequences were analyzed by comparing their nucleotide and amino acid sequence to transcriptome of *B. orellana* the previous reported by Cardenas-Conejo *et al.,* 2015. The results indicated that the sequences obtained are similar to the sequences reported in the transcriptome. Thus, based on the previous results, we optimize the codons of the ORFs in order to express the enzymes involved in bixin biosynthesis in *E. coli* bacteria and thus achieve the production of bixin in a heterologous system.

159. Using molecular markers and chemometric tools to demonstrate that *Clusia suborbicularis* L. is not a synonym of *Clusia flava* J.

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The Mayan jungle, which includes the Yucatan Peninsula in Mexico, is one of the most important biological systems of the world; the Yucatecan flora enlists a wide variety of species, distributed in 956 genera and 161 families. Many of these plants, like those belonging to the Clusiaceae family, are recognized for their ethnomedical uses. To date, four species of the Clusiaceae family are reported to occur in the Yucatan Peninsula: *Clusia flava* J., *Clusia suborbicularis* L., *Clusia rosea* J., and *Clusia lundellii* S., even though *C. suborbicularis* has long been considered a synonym of *C. flava*. The main objective of this investigation was to use molecular markers and chemometric tools to confirm morphological data suggesting that *C. suborbicularis* was a different species. The molecular analysis was carried out sequencing the ITS and EF-1α regions from the leaves and the phylogenetic reconstruction was performed using the Neighbor-Joining method. Chemometric analyses of the HPLC profiles of the bark extracts were performed using PCA and HCA. The results showed differences in the sequence of EF-1α, with a genetic distance of 0.011 %, indicating a common ancestor between *C. flava* and *C. suborbicularis*, while the ITS sequence showed a genetic distance of 0.035 %, confirming that *C. flava* and *C. suborbicularis* are different species. These results were confirmed by both the PCA and HCA analyses of the HPLC profiles of the bark extract, with the former showing three groups, one belonging to those of *C. flava,* another corresponding to those of *C. suborbicularis*, and a third one grouping those of *C. rosea* and *C. lundellii*. The genetic and phytochemical differences between *C. flava* and *C. suborbicularis* can be used to develop chemical markers which could aid in the future differentiation between the two species and other *Clusia* spp.

160. Effects on the content of bioactive compounds in sweet pepper to the open field variety adapted in greenhouse conditions

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Sweet peppers are considered as the best source of ascorbic acid and bioactive compounds, as well as phenolic compounds, which can be improved by the use of inductors. Inductors act as metabolite modulating factors by mimicking stress conditions. Therefore, stress could rarely occur individually, so these conditions could be biotic and abiotic. Then, when the plant is exposed to simultaneous stress processes, it is important to evaluate the effect of inductors such as hydrogen peroxide and salicylic acid, in individual form and mixture, as well as the environmental effect that can be caused by adopting a variety of sweet bell pepper that is normally grown outdoors in greenhouse conditions. Bioactive compounds, antioxidant activity and quality were evaluated. The biotic and abiotic stress evaluated leads to an increase in the evaluated variables ($P \leq 0.05$) when the adaptation stress is applied to the medium and hydrogen peroxide producing more phenolic compounds compared to the control with a 0.023 mg eq. Gallic acid / g sample and 85% inhibition on antioxidant capacity. Regarding the quality, the product was affected to classify within the supreme quality since the length and the polar diameter must be 64 mm respectively and none of the treatments complied with being the closest to this quality the treatment with hydrogen peroxide having 58 mm in length and 64.8 mm in diameter. Therefore, the use of elicitors increased the level of antioxidant compounds, but the second important factor that affected this content was the adaptation of this variety to greenhouse conditions. Therefore, it would be important to continue venturing into this investigation to find the necessary balance to be able to match the quality and content of bioactive compounds.

161. Phytochemical analysis of *Diospyros anisandra* endemic plant of the Yucatan peninsula, of Mexico

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In Mexico, just over 20 species of plants of the genus *Diospyros* are distributed, one of them is *Diospyros anisandra,* endemic specie of the Yucatan Peninsula Biotic Province. Studies on this species are very limited and cover the isolation of compounds that have shown antimicrobial, antituberculous, acaricidal, anthelmintic and antiviral activity. Phytochemical studies on the stem bark showed that *D. anisandra* is a rich source of terpenoids and quinones, among them, plumbagin is a potent inducer of reactive oxygen species (ROS), with anticancer and antioxidant properties. The phytochemical study of this plant is not completed yet, as its leaves, roots and fruits remainds unexplored. In order to contributes to the state of the art of the phytochemical knowledge of this specie we performed the phytochemical analysis of leaves, roots and fruits of this specie.
Plant material were collected to be processed until the dried methanolic extracts of leaf, fruit, stem and root were obtained. Subsequently, the corresponding solid/liquid partitions were carried out for the separation of low, medium and high polarity compounds; proceeding to perform a thin layer chromatography for crude extracts and gas chromatography coupled mass spectrometer for low polarity compounds. The results showed different phytochemical profiles, except for plumbagin that is present in all parts of the plant, thus, we conclude that *D. anisandra* is a rich source of plumbagin.

Key words: plumbagin, *D. anisandra*, metabolite

162. Synthesis of benzyl isoquinoline alkaloids in seed capsules of *Argemone mexicana* L (Papaveraceae)

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Argemone mexicana L. is a medicinal plant producing the benzylisoquinoline alkaloids (BIAs); berberine (protoberberine type) and sanguinarine (benzophenanthridine type). Berberine distribution occurs in all plant tissues, whereas sanguinarine is restricted to roots and mature seeds. Interestingly, berberine could be detected in immature seeds, while sanguinarine is only present at the mature stages. It remains unknown if alkaloids present in the seeds of this plant result from an *in situ* synthesis or they are imported from external tissues. Although sanguinarine could be transported from roots through vascular tissues, it has not been detected in neither stems nor the latex that emanates from it. Alternatively, pericarp could act as the source tissue, since both berberine and sanguinarine share the same intermediaries along the biosynthetic pathway, up to the formation of scoulerine. This work presents a study of alkaloid accumulation in different seed capsule tissues, including pericarp, intralocular septa (ribs) and seeds. Alkaloid accumulation was recorded throughout the complete development process, from the floral buttons up to dehiscent capsule. Berberine was detected in the pericarp and ribs throughout all developmental stages, but only at early seed developmental phases. Conversely, sanguinarine was only detected in seeds, once the desiccation process has started and continuously increasing up to maturation. Distribution of selected transcripts corresponding to early, common biosynthetic reactions, as well as of those leading to either sanguinarine or berberine, suggested that berberine synthesis could occur in external tissues, such as pericarps and then transported to seeds, while sanguinarine formation could actually occur seeds.

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163. Isolation of an ABC transporter tentatively involved in alkaloid mobilization in *Argemone mexicana*

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Argemone mexicana L (Papaveraceae) produces the benzylisoquinoline alkaloids (BIA's), berberine and sanguinarine. Discrepancies among the sites of accumulation of these alkaloids and the transcripts corresponding to biosynthetic enzymes suggest the operation of a long distance transport system for establishing the final alkaloid distribution in mature plants. In fact, in other BIA accumulating species, such as *Coptis japonica* and *Thalictrum minus*, ABC-type transporters, involved in alkaloid mobilization, have been isolated and characterized. A phylogenetic tree was constructed with 479 entries corresponding to putative ABC transporters from an *A. mexicana* transcriptome. Berberine, sanguinarine and auxin transporters from *C. japonica*, *Eschscholzia californica*, *Arabidopsis thaliana* and *Daucus carota* were included as internal and external references. Three candidates, *AmABC-B19*, *-B21* and *-B4*, were selected, based on their association to berberine, sanguinarine and auxin transporters. Candidates for berberine/sanguinarine transporters (*AmABC-B21* and *-B4*) corresponded to partial sequences of 522 and 195 nt, respectively. *AmABCB21* shared a 67 % identity to a 174 residue stretch of the berberine transporter *CjABC-B1* from *C. japonica*, including the transmembrane domain 1 (TMD1), involved in substrate binding. *AmABC-B4* displayed 90% identity to the berberine transporter *TmABC-B2* from *T. minus*, which also presented the TMD1. Therefore, a possible role in alkaloid transport was assigned to them. *AmABCB19* (3744 nt) displayed an 86% identity to the *AtABCB19* auxin transporter form *A. thaliana*. It spanned the complete ORF (1248 residues) and included the transmembrane (TMD1 and TMD2) and nucleotide binding (NBD1 and NBD2) domains. An expression analysis revealed flowers as the main site for transcript accumulation of *AmABCB19*, whereas capsules showed the highest transcript accumulation for *AmABCB4* & *AmABCB21*. These results will be discussed in regard to alkaloid distribution.

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164. Effect of *Callistemon citrinus* on oxidative stress biomarkers in 1,2-dimethylhydrazine-induced colon cancer in rats

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Colon cancer is an important problem in the human health, being one of the main causes of death worldwide. 1,2-dimetylhydrazine (DMH) it is a potent carcinogen metabolized by the liver and excreted into the intestine where the microbiota transforms it into the final carcinogen. Has been shown that many xenobiotics cause an increase in free radicals levels although they usually occur in cells, liver is more susceptible of damage due to its detoxifying function. When reactive oxygen species (ROS) exceed the capacity of the antioxidant system, oxidative stress occurs. *Callistemon citrinus* extract possess a strong antioxidant activity and hepatoprotective effect. The aim of this work was to evaluate the effect of *C. citrinus* leaf extracts on biomarkers of lipoperoxidation cell damage such as malonaldehyde (MDA) and 4- hydroxynonenal (HNE), carbonylate protein and the arylesterase (ARE) activity in livers obtained from Wistar rats with colon cancer. Three experimental groups (n =8) were established: Group 1 (control). Group 2 and 3 received five subcutaneous injections of DMH (65 mg/kg) in the initial three weeks of the experiment. Group 3 additionally, administered *C. citrinus* extract (250 mg/kg) orally every day for 22 weeks. The level of lipid peroxidation (MDA and HNE) and carbonylated proteins were significantly increase and ARE activity decrease in the livers of the DMH treated rats as compared to control animals. *C. citrinus* supplementation to DMH treated rats decreased the lipid peroxidation and the carbonylate protein meanwhile the ARE activity was increase. These results suggest that the extract has a hepatoprotective effect against oxidative damage, in addition to reversing to some extent the damage caused by ROS.

165. Antioxidant capacity in *Stenocereus queretaroensis* cell suspensions

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Given the importance of the roles that antioxidants play in human health, it is now widely recommended to include foods that are rich in these compounds for their beneficial effects on the prevention of cancer and cardiovascular pathologies. Hence, a renewed interest has arisen in the study of new natural sources that can provide antioxidants, such as fruits and vegetables. One example of these new sources is *Stenocereus queretaroensis*, also known as pitaya, which is rich in antioxidants, ascorbic acid, phenols and mucilage, as well as vitamins from the B group, minerals like calcium and phosphorus and a high content of soluble fiber.

In this work, we have determined the antioxidant capacity of two cell suspensions from *S. queretaroensis*, which differ in the group of betalains that they synthetize, whether yellow (betaxanthins) or red (betacyanins). Antioxidant capacity has been measured with the DPPH method that is commonly used for natural and synthetic compounds. Our results point to the fact that red suspensions show higher antioxidant capacity, expressed as □moles TROLOX equivalents g^{-1} FW, as well as a higher concentration of total phenolics, compared to the yellow cell line.

166. HP-TLC analysis of benzylisoquinoline alkaloids in three species of *Argemone*

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The genus *Argemone* (Papaveraceae) includes around 25 species widely distributed in tropical and subtropical areas. These plants produces tyrosine-derived benzylisoquinoline alkaloids (BIA´s), some of them with pharmacological uses. Berberine (protoberberine) and sanguinarine (benzophenatridine) have been identified in tissues of different *Argemone* species. However, despite the biosynthetic potential of this genus, a systematic phytochemical study have not been performed yet. In order to analyze the diversity of alkaloids, three *Argemone* species, representative of Mexico´s diversity, were selected: *A. mexicana* L *A. platyceras* L and *A. ochroleuca* Sweet. Extracts from leaves, stems and roots were obtained and BIA profiles were analysed by thin-layer chromatography (TLC) and high-performance-TLC (HP-TLC) An HP-TLC method, that allows the generation of unique alkaloid profiles, high reproducibility and great separation power was developed and validated by coupling HP-TLC to *in situ* densitometry. The contents of berberine and sanguinarine in extracts from the different tissues could analyzed, obtaining reliable and reproducible analytical results. The effective separation of the components present in the alkaloidal extracts was achieved using two elution systems of high and medium polarity. Besides sanguinarine and berberine, cheleritrine and dihydrosanguinarine were also identified.
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167. Identifying genes involved in betalain synthesis in *Stenocereus queretaroensis*

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Betalains are hydrosoluble nitrogenous compounds, formed from a central chromophore of betalamic acid, which derives from tyrosine. These compounds are restricted to the Caryophyllales order and present colors that range from yellow (betaxanthins) to purple (betacyanins). Given present restrictions to use synthetic colorants, interest in these pigments has increased due to their stability under several manufacturing conditions (pH, heat, etc.) and to their diverse bioactive properties. Although many studies have focused on the chemistry and synthesis of betalains, as well as on the physiological aspects through potential experimental models, their biosynthetic pathways have not been completely elucidated. *Stenocereus queretaroensis* is an arboreal cactus, whose ovoid fruits (pitayas) are able to synthetize betalains. Pigments extracted from these fruits present some advantages compared to those from other sources, such as beetroot and Swiss chard, regarding residual flavors or odors when processed. Using the new generation of sequencing technologies, like RNA sequencing (RNA-seq), our aim is to obtain the transcriptomes from two *S. queretaroensis* cell lines of contrasting colors: SqY1 and SqR1, which produce betaxanthins and betacyanins, respectively, in order to identify key genes involved in betalain synthesis and to study their expression patterns and their enzymatic products.

168. Preliminary phytochemical analysis of the moss *Physcomitrella patens*

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Physcomitrella patens (*Physco*) is a moss that possess a sequenced genome[1], which allows genomic and proteomic studies. Transcriptomic analysis of Physco revealed that there are many gene products involved in secondary metabolism, but the information about this issues are very scarce. Erxleben et al (2012)[2], showed that Physco harbors osmoprotective molecules that were conserved during land plants evolution. But more studies are required to increase the knowledge about its metabolic composition, not only for the understanding of the biological system, but even for his commercial value and medical applications. In the present work, protonemata tissue of *Physco* was sub-cultivated every week in liquid plain mineral medium, under 16/8 hr photoperiod at 22°C and 200 rpm shaking. Tissue was homogenized and filtrated with a polypropylene gauze (250 µm). Once the plant tissue was recollected, 13 g of fresh material was lyophilized. The qualitative phytochemical analysis of the crude powder of the protonemal tissue collected was determined following the methodology proposed for Aiyelaagbe et al (2007)[3]. These first results revealed that the aqueous extract of protonemal tissue of *Physco* contains phenols, poliphenols, reducing sugars, alkaloids, flavonoids and saponins, in different proportions. These first analysis show us the important antioxidant activity of the extract derived from the protonemal tissue of the moss *Physcomitrella patens*. This is our first step into the use of the moss as a source of extracts with metabolites for the synthesis of nanoparticles with potential use in several areas of biotechnology.

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169. Effect of *Callistemon citrinus* (Curtis) Skeels on oxidative stress biomarkers in rats fed a high fat diet

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Lipid catabolism in the β-oxidation followed by the electron transport chain have been identified as the main factor involved in the formation of highly oxidizing products during the consumption of hypercaloric diets. In *C. citrinus*, compounds with a potent antioxidant capacity have been identified, 1,8-cineole, limonene and α- terpineol have been recognized as chemotherapeutic agents in some diseases.

The aim of this study was to evaluate the effects of normal and high-fat diet supplemented with *C. citrinus* leaf extract on lipid peroxidation, carbonylated proteins and glutathione reduced in the heart and liver of Wistar rats. 24 male albino Wistar rats were randomly divided into four groups (n=6). Group 1 served as control, groups 2 was group hypercaloric diet (HFD), group 3 was HFD plus simvastatin (3 mg/kg) and group 4 received HFD plus leaf extract of *C. citrinus* (250 mg/kg).

The results showed that high-fat diet alone caused lipid peroxidation (MDA and HNE) increased in the liver and heart, the level of protein carbonyl were significantly different in the liver and heart, while in both organs the GSH levels decreased. On the other hand, the administration of simvastatin and *C. citrinus* extract decreased the formation of these oxidative products and the level of GSH was similar as the control group. These results suggest that C*. citrinus* suppress oxidative stress and protect the liver and heart in rats.

170. Chromatographic profile of fractions with anti-hemagglutinin activity obtained from the stem bark of *Caesalpinnia yucatanensis*

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Caesalpinia yucatanensis is a tree widely distributed in the Yucatan Peninsula, where is used in traditional Mayan medicine to treat fever, headache and diarrea. Plants belonging to the genus Caesalpinia have shown antiviral activity against influenza virus, as is the case of *C. yucatanensis*. The hexane fractions obtained from the stem bark of this specie showed antiviral activity at the co-treatment level against two strains of influenza A virus, sensible (AH1N1pdm09) and resistant (AH1N1 INDRE). Therefore, it is believed that one or more components present in the stem bark of *C. yucatanensis* could be inhibiting the binding of the hemagglutinin viral protein to cell receptors. For the present project, 22 kg of fresh stem bark of *C. yucatanensis* were collected, ground and its methanolic extract (MeOH) (2.5%, 4.4%) was obtained. The MeOH extract was partitioned by a liquid-liquid separation, the low polarity extract (hexane extract) obtained was fractionated by vacuum liquid column chromatography (CLV) with the help of thin layer chromatography (CCD). The fractionation was bioguided by the hemagglutination inhibition (IHA) test. Fractions with anti-HA activity were analyzed by HPLC / MS in order to elucidate the nature of the active(s) ingredients. KEY WORDS: antivirals, influenza A, anti-hemagglutinin.

171. Increase of flavonoids in *Capsicum chinense* fruits during plant infection by *Pythium ultimum* as part of systematic acquired response

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The phytopathogen oomycete *Pythium ultimum* is capable to infect a wide variety of plant hosts. As a defense mechanism plants response with the induction of several secondary metabolites such as phenolic compounds that have an impact in plant adaptation process to their environment. The objective of this work was to evaluate the induction of flavonoids in fruits of habanero pepper (*Capsicum Chinense* var. Chichen Itza) during the infection of the plant through the roots by *P. ultimum.* Flavonoids of habanero pepper fruit extracts obtained from infected and not infected plants were identified and quantified by ultra-high efficiency liquid chromatography (UPLC) with a photodiode detector (PDA) and mass spectrometry (MS). The results indicated an increase of 74.59% and 84.21% of luteolin-7-O-(2-apiosyl-6-malonyl) hexoside and apigenin-7-O-(2-O-apiosyl) hexoside respectively, in fruits of plants infected. Induction was compared with the genetic expression of enzymes related to the flavonoid biosynthetic pathway. The increment of flavonoids in the fruit is suggested as a part of a systematic acquired response (SAR) as a mechanism of protection against the possible attack of *P. ultimum*. According to some authors apigenin and luteolin derivates are capable to inhibit spore germination and are involved in the formation of mechanical barriers that prevent the progression of the infection.

172. Evaluation of total phenolic content and phenolic profile (free and bound compounds) of *Byrsonima crassifolia* fruit by HPLC-PDA-MS

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Byrsonima crassifolia known as “nanche” is a popular tropical fruit distributed in Mexico and South America. This is known as an important source of phenolic compounds. The objective of this work was to evaluate the total phenolic content and profile of free (FPC) and bound (BPC) phenolic compounds in *B. crassifolia* fruit. FPC and BPC were extracted sequentially by maceration and alkaline hydrolysis, respectively. Total Phenolic Content (TPC) was determined by Folin-Ciocalteu assay and phenolic profile by high performance liquid chromatography (HPLC) with a photodiode array detector (PDA) and mass spectrometry (MS). The results showed a concentration of 12.26 ± 0.03 mg GAE/g DW of FPC, and 17.58 ± 0.31 mg GAE/g DW of BPC. This result suggests that phenolic compounds in *B. crassifolia* fruits are principal associated to cell wall structures or other cellular components. HPLC results indicated that the principal flavonoids in FPC extract were identified as quercetin-3-O-galactoside and quercetin-3-O-xyloside, while in BPC extract flavonoids and anthocyanin derivatives were detected. Several plants produced preferentially quercetin derivatives due to its capability to act in different adaptative functions as in response to oxidative and light stress. Regarding BPC their functions are poorly understanding. Besides, it is suggested that they could have a mechanical or photoprotective function in fruit development.

173. Biochemical changes in *Agave americana* L. plantlets induced by ethyl methanesulfonate

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A. americana L. is a crop with very little genetic variability [1]. Ethyl methanesulfonate (EMS) is the most commonly used chemical mutagen in plantlets [2]. In order to evaluate the effect of ethyl methanesulfonate (EMS) in *in vitro* plantlets of *A. americana,* callus were treated with 15 mM EMS for two hours after which shoot formation was induced using 2,4-D (0.11 µM) and BAP (44 µM). The parameters were measured in leaves of acclimatized plantlets, EMS-treated and control, which included photosynthetic efficiency (Pe), nitrogen content, anthocyanin (Anth) and flavonoids (Flav) using the Dualex sensor (FORCE-A, Orsay, France) according to [3]. In addition, chlorophyll fluorescence was measured by a Chlorophyll Fluorometer according to [4]. The method of Beaudoin-Eagan & Thorpe [5] was used to estimate PAL activity. Liquid Chromatography (HPLC) was used for the quantification of the carbohydrates (sucrose, glucose, fructose and fructans). Fructans and fructose concentrations were higher in plantlets from callus exposed to EMS in comparison with control plantlets ($p<0.05$), whereas sucrose and glucose concentration were not significantly different. Studies have shown that the use of EMS increases the genetic variability of plantlets thereby overcoming some agronomic and environmental problems [6]. It was observed that photosynthetic efficiency and flavonoid content did not present significant differences between control plantlets and those treated with EMS. Anthocyanins and PAL activity were higher in the plantlets treated with EMS. The resulting increase of anthocyanins concentration and PAL activity in plantlets treated with EMS could be explained by the fact that anthocyanins are flavonoids formed by phenylpropanoid metabolism from phenylalanine. The results indicated that the EMS caused increased the fructan and fructose content by 30%. PAL was increased and this activity is related with higher anthocyanins concentration in *A. americana* L. plantlets.

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174. Metabolic responses in whitefly infested and newly developed systemic leaves of husk tomato (*Physalis philadelphica*)

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The resistance of plants to herbivory attack depends on changes in their metabolic reserves to improve tolerance or defense. These changes occur both in local herbivorized and in systemic undamaged tissues. The occurrence of metabolic changes in systemic tissue are thought to evolved as a priming mechanism to counteract future herbivore attacks by preparing yet undamaged systemic tissue for enhanced resistance. However, these metabolic responses have been found plant- and herbivore- specific. This research aims to analyze metabolic fingerprinting of local and newly developed systemic leaves of *P. philadelphica* plants after whitefly (*Trialeurodes vaporariorum*) infestation and compare their metabolic differences. Using UPLC-MS, we obtained untargeted metabolic profiles of local (L) and systemic leaves (S) of whiltefly-herbivory (-H) and undamaged control (-C) plants and analyzed them using multivariate statistical (MVS) tools (PCA and O/PLS-DA).

For all treatments and tissues, a total of 216 features were obtained (positive and negative mode). MVS analyses of metabolic profiles indicated significant differences among plant tissues and herbivory condition, with the strongest differences found between S-H to L-H and S-C. Metabolic signature abundance was higher for whitefly-herbivory plants in S tissue. After herbivory, we found an increase (>3 fold) in the abundance of 20 mz features in L and 134 in S tissues, and a decrease (<0.5) in 32 and 16 mz features in L and S tissues, respectively.

Interestingly, from the (134) features increased in S tissue of whitefly-herbivory plants, 58% were found more abundant in L than in S tissue of undamaged C plants and changed to a higher abundance in S after whitefly-herbivory. This finding highlights an important metabolic reconfiguration of the S tissue after whitefly-herbivory. Further studies will address biological relevance of this metabolic reconfiguration to preexisting systemic tissues during the herbivore attack.

175. Immunodetection and Enzymatic Activity of Cytosolic ADP Glucose Pyrophosphorylase in Banana pulp (*Musa acuminata*)

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Adenosine diphosphate glucose pyrophosphorylase (AGPase) is the enzyme that produce ADP-glucose in the first step of starch biosynthesis pathway. Starch accumulation level is associated with the crops yield. In the main food crops, the AGPase is found in amyloplast (tubers, legumes, roots). However, in cereals the AGPase is present in amyloplast and cytosol. In the maize endosperm, the cytosolic AGPase represents 90% of the total activity of the enzyme. In banana pulp was reported that the AGPase activity increased according to the development of the fruits but was not specified the form (cytosolic or amyloplastidial). The objective of this work was identified the AGPase in banana pulpa by inmmunodetection and determine its activity to know also is present in cytosol. Banana fruit of three development stages (11, 16 and 21 weeks after flowering, *waf*) were collected; the protein extracts from cytosol and amyloplast were obtained and analyzed. Protein content increased between the 11 and 16 *waf*, but at 21 *waf* the protein levels decreased 50-70%. The total, cytosolic and amyloplastidial extracts of the 16 *waf* had the highest amount of protein. There was always higher protein content in the cytosol than in the amyloplast. A major band was located between 50 and 55 kDa. The immunodetection of *Musa acuminata* AGPase corresponds to the molecular weight (Mw) reported for the small subunit of the AGPase in maize and rice. AGPase activity increased from 11 to 16 *waf*, followed by a decrease at 21 *waf*. The activity of the cytosolic isoform is higher than that of the amyloplastidial isoform. Cytosolic AGPasa in banana pulp is responsible of the high activity for the production of ADP-glucose to sustain the starch synthesis.

176. *Critonia aromatisans* & *Plectranthus amboinicus* extracts: new possible miticides alternatives against the Honeybee (*Apis mellifera*) parasite *Varroa destructor*

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Due to the risk that the mite *Varroa destructor* represents for beekeeping worldwide and the disadvantages of using synthetic acaricides, it is essential to find natural origin alternatives to control it under an integrative point of view. In this sense, *Lonchocarpus punctatus*, *Cymbopogon citratus*, *Plectranthus amboinicus* and *Critonia aromatisans* are plants species with distribution in the Yucatán Peninsula, with reported secondary metabolites in their composition such as stilbenes and isoprenoids which usually have acaricidal activity, so its extracts may present activity against this parasite. In this work the acaricidal activity of the low polarity extracts from *L. punctatus*, *C. citratus*, *P. amboinicus* and *C. aromatisans* through an acute toxicity test (ATT) was evaluated. In addition, their effect on *Apis mellifera* (contact toxicity test, CTT) and their associated microbiota (disc diffusion and microdilution test) (*Candida apicola*, *Candida versatilis*, *Kurtzmaniella cleridarum* and *Zygosaccharomyces mellis*) were verified. GC-MS analysis was also performed on all extracts. In the ATT *C. aromatisans and P. amboinicus* showed 100% mortality at the doses of 200 and 150 µg, respectively. Likewise, these extracts caused the highest mortality in the CTT test and the lowest MIC on the yeasts sensitivity evaluation, after thymol and amitraz. GC-MS analysis showed the presence of carvacrol and cyclocolorenone among the majority compounds in the extract of *P. amboinicus* and *C. aromatisans*, respectively. In spite of the obtained results, these two extracts should not be eliminated as an alternative of treatment against *V. destructor*, since this work provides us with the bases to identify the most appropriate way to apply a treatment with these extracts. In addition, these results support the carry out of a bio-guided isolation assay.

177. **Alkaloid synthesis during *in vitro* morphogenesis in *Argemone mexicana* L.**

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Cotyledons and leaflets of developing seedlings of *Argemone mexicana* (Papaveracea) simultaneously accumulate the benzylisoquinoline alkaloids berberine and sanguinarine. However, in leaves from mature plants only berberine can be detected. Both alkaloids are present in roots, regardless their developmental phases. Upon induction of *in vitro* cultures from leaves the levels of berberine and sanguinarine levels displayed opposing trends. Berberine steadily decreased from the initial explant, up to the proliferation of disorganized cell masses, while sanguinarine content continuously increased. Once callus cultures were established, sanguinarine was the primary alkaloid present, whereas berberine could not be longer detected. Rootless shoots regenerated from these calli presented both berberine and sanguinarine. However, once roots were formed, sanguinarine was relocated to this organ and berberine was evenly distributed in both organs. When internode explants were used, axillary shoots emerged directly from lateral buds, without callus formation. Both berberine and sanguinarine were observed. No root formation was observed in this case. Therefore, alkaloid synthesis in *in vitro* cultures of *A. mexicana* is related to tissue organization and berberine, but not sanguinarine, accumulation requires the presence of differentiated organs. Transcript accumulation of berberine and sanguinarine related biosynthetic genes was followed during the process, confirming this interpretation.

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178. Effect of methyl jasmonate on the biosynthesis of iridoids and phenolic compounds in *Castilleja tenuiflora* Benth

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Castilleja tenuiflora Benth. (Orobanchaceae) is a Mexican plant of biotechnological interest to produce phenolic compounds and iridoids with therapeutic potential. Methyl jasmonate (MJ) is a signal molecule in the biosynthetic pathways of secondary metabolites, regulating the transcription of genes involved in its biosynthesis. The objective of this study was to evaluate the effect of MJ on the accumulation of chemical compounds and on the transcriptional activation of genes involved in the biosynthesis of secondary metabolites of *C. tenuiflora*.

Thirty-day-old *in vitro* plantlets of *C. tenuiflora* were treated with 100 µM MJ; the dynamic accumulation of the majority compounds (iridoids; aucubin and phenolics; verbascoside) was evaluated, as well as the transcriptional activation of the genes encoding the enzymes: phenylalanine ammonium lyase Cte-pal; chalcone synthase Cte-chs; deoxyxylulose-5-phosphate synthase Cte-dxs; and geraniol-10 hydroxylase Cte-g10h; after elicitation (1, 2, 4 and 7 days).

The highest accumulation of aucubin was observed one day after elicitation, however, transcriptional activation of Cte-dxs and Cte-g10h was observed four days later. On the other hand, the highest accumulation of verbascoside was observed seven days later, which was accompanied by the transcriptional increase in Cte-pal and a decrease in Cte-chs levels. The behavior of the transcription levels showed a positive correlation with the biosynthesis of verbascoside, however, no correlation was showed for aucubin.

It can be concluded that the MJ triggers protective responses with respect to time in the plantlets of *C. tenuiflora* after elicitation. The activation of the biosynthesis of the metabolites can be defined as "early" with the synthesis of iridoids and "late" with the synthesis of phenols. MJ can help modulate the rate of transcriptional initiation of these genes, promoting the adequate accumulation of secondary metabolites, controlled spatially and temporally.

SIGNAL TRANSDUCTION

179. Characterization of plant innate immunity using the early response gene *ATL2* as a model.

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The early response genes are an important part of the responses activated by the plants against pathogens, since they participate on the induction and regulation of the defense mechanisms. However, the molecular elements involved in their regulation are not complete known. 5 mutants, called *eca*, have been isolated because they constitutively express the PAMP-induced early response gene *ATL2* (Serrano & Guzmán, 2004). Recently, it has been determined that the *eca2* mutant is resistant to the fungus *Botrytis cinerea* and the bacterium *Pseudomonas syringae*, in addition to that, the permeability of the cuticle is increased in *eca2* due to the decrease in the content of waxes and cutin monomers (Blanc *et al*., 2018). In order to identify molecular elements that intervene in the regulation of *ATL2* mediated by *ECA2*, we performed a genetic screening to identify *reca* mutants, which are revertants of the constitutive expression of *ATL2* in the mutant background *eca2*. *reca1* was isolated and it shown different phenotypes, including a reduction of the rosette size, as well as an increase in the number of leaves. The *reca1* is susceptible to *B. cinerea* but resistant to *P. syringae* and has a more permeable cuticle compared to the wild type plant. It was determined that the phenotype of *reca1* is mediated by a single recessive and independent gene of *eca2* mutation. Therefore, *RECA1* could be acting as a new molecular element, which positively regulates the expression of *ATL2*, which according to the phenotypes evaluated in *reca1*, are not completely reverted to those observed in the wild type plant, but in many of the cases, *reca1* presented intermediate phenotypes between the wild plant and the *eca2* mutant. In this work we will present the genetic and transcriptomic characterization of the mutant *reca1,* that might allow us to elucidate the molecular mechanisms that intervene in the regulation of early response genes.

180. New mechanisms of activation and inhibition of maize CDKs

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Cyclin dependent kinases (CDKs) are proteins that, associated with their regulatory subunits, the cyclins, phosphorylate a high number of substrates during the different stages of the eukaryotic cell cycle, allowing its progression. In general, CDK activity is regulated by positive or negative phosphorylation, mediated by very conserved protein kinases. Activating phosphorylation is performed by CDK activating kinases (CAK), and the inhibitory phosphorylation is mediated by Wee1 kinase.
We have previously found the *in vivo* phosphorylation of CDKs, however, we could not observe the expected correlation between kinase activation and CDK phosphorylation, therefore in this work we have used an *in vitro* system to study the effect of both inhibitory and activating phosphorylations on maize CDKA1 and CDKB1;1. Activation of both CDKs, as expected, requires their association to cyclins; activity of CDKA1 was notably increased after phosphorylation by *S. cerevisiae* CAK, on the other hand and unexpectedly, CDKB1;1 associated to a cyclin was autophosphorylated in an aminoacid residue that is recognized by an antibody developed against the activating phosphorylation sequence, suggesting that this CDK does not require a CAK for activation.
When the Wee1 kinase was used to study its ability to phosphorylate and inhibit CDK activity, it was found that maize Wee1 barely phosphorylated CDKA1, but CDK kinase activity was strongly reduced; additionally, a catalytically inactive Wee1 mutant was able to inhibit CDK activity. We show that Wee1 associates to CDKA1, and suggest that this association is sufficient to inhibit CDKA1 activity, constituting a new mechanism of CDK regulation by Wee1.

181. Stress effect by aluminium in phospholipases C behaviour from *Coffea arabica* L.

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Adequate signal transduction pathways in plants determine their successful adaptation to diverse biotic and abiotic factors. Our group employed suspension cultured cells to study the phosphoinositide pathway, which is triggered by aluminium stress. We investigated members of the PI-specific phospholipase C (PLC) family and evaluated their transcription profiles in *Coffea arabica* suspension cells after 14 days of culture when treated or not with 100 µM $AlCl_3$. The four *CaPLC1-4* members showed changes in their mRNA abundance upon $AlCl_3$ treatment. The expression profiles of *CaPLC1* and *CaPLC2* exhibited a rapid and transitory increase in abundance. By contrast, *CaPLC3* and *CaPLC4* showed specific profiles that were down- and upregulated, respectively. CaPLC proteins were heterologously expressed, and CaPLC2 and CaPLC4 were tested for *in vitro* activity in the presence or absence of $AlCl_3$ and compared to *Arabidopsis* PLC2 (AtPLC2) and a crude extract isolated from coffee cells. CaPLC2 showed a similar inhibition (30%) as AtPLC2 and the crude extract, while CaPLC4 activity was enhanced by $AlCl_3$. Additionally, we visualized the YFP-$PH_{PLC\delta1}$ subcellular localization in cells that were treated or not with $AlCl_3$. In non-treated cells, we observed a polar fluorescence signal towards the fused membrane. However, when cells were treated with $AlCl_3$, these signals were disrupted. Finally, this is the first time that PLC activity has been shown to be stimulated *in vitro* by $AlCl_3$. Therefore, a relevant role for PLC in the signal-transduction response to aluminium stress can be established, reflecting a co-evolution strategy for *Coffea* species to survive in adverse aluminium acclimations.
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182. The role of TOR signaling pathway in the oil-producing microalga *Botryococcus braunii* race B

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The colonial green microalga *Botryococcus braunii* race B produces, accumulates and secretes large amounts of long-chain liquid hydrocarbon oils (triterpenoids C30-C37) called botryococcenes that is typically in the range of 30-50% of algal dry weight. Its hydrocarbons can be used as renewable feedstock for producing petroleum-equivalent fuels suitable for combustion engines, using the existing oil refineries. However, one of the main constraints for its commercially feasible use is that *B. braunii* race B is a slow growing microalga with a cell doubling time of 6 days. It is hypothesized that its slow growth, could be due to the large amount of photosynthates invested for the biosynthesis of hydrocarbons via the methylerythritol phosphate (MEP) pathway at the expense of biomass production. The study and manipulation of signaling pathways regulating cell growth and metabolic processes in microalgae, such as the target of rapamycin (TOR) pathway, may help to overcome the balance between biomass productivity and terpenoid or lipid yield. Therefore, in the present work we will seek to study the physiological, metabolic and molecular response of the microalga *B. braunii* race B (Showa strain) to the pharmacological inhibition of the TOR kinase activity with rapamycin or AZD-8055, as well as its activation with glucose and auxin.

183. Unravelling the genetic mechanisms by which titanium acts as a beneficial element in plants

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Treatment with titanium has a beneficial effect on crops by enhancing nutrient absorption and photosynthetic rate, leading to improved yield and higher quality. The physiological effects of Ti in plants have been explored previously, but the genetic mechanisms and the components involved in the response of plants to this element have not been identified. Interactions between Ti and essential elements have been observed, among them is phosphorus, it is known that Ti application can amend inorganic phosphorus (Pi) deficiency. It has been suggested that this effect can be due to an enhanced production of malate and citrate in Ti treated plants, that is a common response of plants to the Pi deficiency. The local low Pi response involves a malate-dependent Fe accumulation and remobilization that modifies root architecture. Moreover, It is also believed that Ti can interact with components of the signaling transduction pathways of Fe and could also interfere with Fe-dependent redox reactions or electron transfer reactions. We characterize the response of plants under low Pi supplemented with Ti. Our results suggest that Ti is capable of initiating the same signal transduction of Fe that inhibits the root growth but by a different mechanism. With this knowledge we started a screening with an EMS-induced mutant population to identify mutants unable to respond to titanium treatment. So far we have identified 10 mutants with verified phenotypes to be defective to response to Ti treatments. Further characterization of these mutants and the advances in mapping the genes resposible for the observed phenotypes will be reported.

184. Study of AIB and MYB122 transcription factors in glucose signaling in *Arabidopsis thaliana.*

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Sugars act as signaling molecules modulating the metabolism, physiology, growth and development of the organisms. Plants are autotrophic organisms and therefore, perception of sugar levels is critical for the production and management of this energy source. Further, sugars regulate fundamental processes during the growth and development of plants at genetic level through signaling pathways. Despite the importance of sugars as signaling molecules in the regulation of gene transcription, the current knowledge of the components and the molecular mechanisms of signaling pathways is still limited, such as the case of the last effectors, the transcription factors. In order to establish new components in sugar signaling pathways, the study of the regulation of *STP1* gene by glucose has been addressed in *Arabidopsis thaliana*. *STP1* encodes a high affinity hexose transporter, and its expression is repressed by glucose, involving a hexokinase 1 independent pathway. Previously, by a DNA Pull-down assay, we identified the transcription factors AIB and MYB122 as possible regulators of the *STP1* expression in response to glucose, via directly interacting with regulatory *cis* element(s) located in a delimited region of 310 bp of *STP1* promoter. In order to determine the participation of AIB and MYB122 in glucose responses, we evaluated the phenotype of *aib* and *myb122* mutants during the germination and early plantlet development in presence of different glucose concentrations. This analysis demonstrated that both mutants presented an altered development in low glucose concentrations, showing a hypersensitivity phenotype, in comparison to wild type plants. Also the skotomorphogenic development was analyzed and both mutants showed shorter hypocotyls and an altered accumulation of starch with respect to wild type plants. This first approach suggests that AIB and MYB122 participate in glucose signaling, regulating early developmental processes.

PLANT STRESS RESPONSE TO ENVIROMENT

185. Characterization of phenotypic, physiological and expression levels of trehalose coding genes in maize seedlings under drought stress.

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In plants, trehalose has a vital role as a mediator of different abiotic stress such as drought, high and low temperatures and osmotic stress. Thus, the role it plays in adapting plants to various stresses has been widely documented. The aim was to characterize the phenotypic, physiological changes and expression levels of the trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) genes in maize seedlings under drought stress. For this, resistant and susceptible maize genotypes (CML 551 and CML 311) were subjected to water stress by means of irrigation retention for 72 hours. The results showed that the drought reduced the amount of leaves, the height and the weight of the wet and dry mass of the plants. In resistant plants (CML551) there was an increase in the Concentration of Photosynthetic Pigments (CPSP), proline content and invariability to oxidative stress (SOD). The susceptible plants (CML 311) showed a decrease of CPSP and oxidative stress damage. Concerning the quantification of the TPS and TPP genes expression, high levels of expression of both genes were observed in resistant plants compared to those susceptible at different times. The results suggest that maize plants under drought tolerance can use many strategies and mechanisms to mitigate damage such as trehalose production.

186. A novel phosphorylated residue implicated in the activation of SnRK1

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SnRK1 is a protein kinase complex involved in the tolerance to different adverse conditions by regulating energy homeostasis and metabolism. SnRK1 consist of one catalytic subunit (α), and two regulatory subunits (β and γ). Activation of the catalytic subunit depends on the phosphorylation in a conserved Thr present at the activation loop by activating kinases (SnAKs). The mechanism of action postulated for these kinases begins with the activation of SnAKs by self-phosphorylation. Once activated, they phosphorylate the α-type subunits of the SnRK1, increasing their activity. The SnRK1 phosphorylates back the SnAK at an inhibitory site. Even though it is accepted that phosphorylation in the Thr present at the activation loop indicates activation of the SnRK1, not always this modification correlates with the kinase activity. We studied the activation and phosphorylation of the catalytic subunits using different mutants of the catalytic domain (DC-SnRK1α). We found that another phosphorylation site was also crucial for SnRK activity. In this work, we discuss the importance of this finding in terms of SnRK1 activation.

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187. Overexpression of fructan exohydrolases in *Arabidopsis thaliana*

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Fructans are polymers of fructose synthesized by approximately 15% of higher plant species which are degraded by fructan exohydrolases (FEHs). In the *Arabidopsis thaliana* genome, six putative cell wall invertases *(AtcwINV1-6)* were annotated some of which have been funtionally caracterized by heterologous expression in *Pichia pastoris*, revealing that *AtcwINV3* and *AtcwINV6* are FEHs and not invertases as previously believed. Furthermore, there are reports where FEH enzymes have been identified and characterized in biological systems that naturally do not synthesize fructans such as *Beta vulgaris, Nicotiana tabacum* and *Rumex dentatus*. Although, their function is poorly elucidated, it has been suggested that these FEHs might have activity related with cold tolerance and defense by acting on bacterial fructan forming biofilms. To analyze their biological function, the cDNAs of *AtcwINV3* and *AtcwINV6* were cloned and overexpressed in *A. thaliana* lines by using the binary expression vector pB7WG2D. The genetic transformation was mediated by *Agrobacterium tumefaciens* GV2260 strain through floral dip. By examinating the Arabidopsis EFP browser database, higher expression of *AtcwINV3* in floral tissue was observed whereas *AtcwINV6* was highly expressed in vegetative roots. To verify the integration of the T-DNA, a PCR was carried out to amplify the *BAR* gene. Until now, 6 homozygous lines have been generated for the overexpression of *AtcwINV6*. The overexpression lines of *AtcwINV3* are still under selection in order to obtain homozygous lines. Phenotypic analysis of the *AtcwINV6* lines showed significant differences in root length in comparison to the wild type control. This result is consistent with the *in silico* expression pattern and suggests that *AtcwINV6* may be involved in root system development.

Key words: *A. thaliana,* fructan*,* fructan exohydrolases, overexpression.

188. Functional characterization of group 6 LEA proteins in *Arabidopsis thaliana*

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Plant responses to water deficit involve complex signaling networks leading to plant tolerance and adaptation. Among the conserved plant responses to this stress condition is the accumulation of Late Embryogenesis Abundant (LEA) proteins, whose levels increase in response to water limitation during plant development, in dry seeds, in pollen grains, and in vegetative tissues under water deficit imposed by the environment. These observations have led to the hypothesis that LEA proteins participate in the plant tolerance to this environmental stress. To get insight into the role of these proteins in the plant response to water deficit, we have focused in group-6 LEA proteins, a highly conserved LEA protein family. In this work, we present data showing that AtLEA6-1 protein absence leads to sensitive phenotypes under osmotic (350 mM mannitol) and salt (250 mM NaCl) stress. We further showed that salt sensitivity persists during seedling establishment, and that the lack of this protein also affects root development. We confirmed that the salt sensitive phenotype resulted from AtLEA6-1 absence by complementation assays with the wild-type gene. Noteworthy, *atlea6-1* mutant seeds also show lower germination rates than wild-type seeds under non-stress conditions, suggesting that the lack of this protein may be affecting seed dormancy and/or desiccation tolerance. Altogether, these data show that AtLEA6-1 protein is involved in the plant adaptive response to low-water availability and that its function is not redundant with LEA proteins from the same and other groups. The possible function(s) of LEA6 proteins will be discussed.
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189. Advances in the functional analysis of two genes of the AP2 family of Physcomitrella patens involved in ABA, osmotic stress, and glucose responses

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In *A. thaliana*, AP2 transcription factors participate in the regulation of several processes such as development, abiotic stress, responses to ABA and glucose, among others (León et al., 2013; Wind et al., 2012). Many stress-responsive transcription factors have been identified and studied in vascular plants, however, just a few have been analyzed in non-vascular plants. Our research group identified two putative AP2-like genes in the moss *Physcomitrella patens*, and several pieces of evidence suggest that both could be involved in the responses to ABA, abiotic stress and glucose. To explore the regulation of the expression of such genes at the tissue level, we generated stable transcriptional fusion lines for both genes by substituting each gene with *gfp* gene, generating at the same time individual knock-out mutants. Phenotypical analysis of these lines revealed that both mutants show a lower sensitivity to high glucose concentrations. In contrast, both transcriptional fusion lines present a higher sensitivity to ABA. These findings strongly suggest the participation of both AP2-like genes in the regulation of ABA and Glc responses in *P. patens*. Furthermore, to confirm the subcellular localization of their products, we obtained translational fusions lines by the substitution of the stop codon of both AP2-like ORFs with the ATG of *gfp* gene.

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190. Identification of transcripts of Glutamate Receptors in ontogenetic stages of flowers and fruits of habanero pepper (*Capsicum chinense* Jacq.) under different environmental conditions

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The ionotropic glutamate receptors (iGluRs) are integral membrane proteins that were initially discovered in mammals and whose main function is to carry out most of the excitatory neurotransmissions in the central nervous system (CNS). This type of proteins has been found to function in learning, memory and the development of neural networks. Dysregulation of these proteins can lead to acute neurological disorders, such as epilepsy and Parkinson's. Surprisingly, analogous sequences to iGluRs have been found in other organism's genomes that do not possess a CNS, such as bacteria and plants. The sequences identified in the genome of the plants were called GLRs (glutamate-like receptors). Among the plant species where these proteins have been identified are tomato, rice, radish, *Arabidopsis thaliana* and *Echinochloa cruz-galli*, being those of *A. thaliana* the most studied. Published data suggest that GLRs participate in several biological processes of plants, such as reproductive development, carbon-nitrogen balance, the response to water stress, among others. The aim of this project was to study the *Capsicum chinense* GLRs (CcGLRs) possible participation during the floral and fruit ontogeny of this specie, through their transcript profiles during these processes. The results showed that the expression of 12 CcGLRs were dependent on the type of GLR and the stage of development. Also, it was observed that the levels of transcripts of some of these genes are modified when the plants are subjected to different nitrogen doses and under conditions of water deficit. These results give new insights and establish new ways in the study for the elucidation of the function of these proteins in this plant specie.

191. Revealing LEA protein-protein interactions to understand protein stabilization under stress conditions

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Plants as organisms of sessile nature are exposed to stress conditions that affect their distribution, growth, and development. Natural selection has driven the evolution of sophisticated physiological and molecular responses to stress. Members of the late embryogenesis abundant (LEA) protein family represent highly hydrophilic and intrinsically disordered molecules that acquire structure upon dehydration. The accumulation of LEA proteins in response to water deprivation strongly suggests that these proteins are determinants of stress tolerance. *In vitro* assays have demonstrated the ability of several LEA proteins to prevent enzyme inactivation during freezing and drying. Also, the over-expression of LEA genes can improve plant stress tolerance. Despite the importance of LEA proteins in stress responses, it is not understood how these proteins can stabilize enzymes during dehydration. Our work aims to elucidate protective mechanisms of LEA proteins through the study of protein-protein interactions. Over-expression of the *OpsDHN1* gene, a member of the LEA_2 group (Dehydrin; DHN) from cactus pear, enhances Arabidopsis freezing tolerance. The OpsDHN1 protein is able to interact with itself and with the three Arabidopsis DHNs COR47, ERD10, and RAB18 *in vivo*. Our analyses also revealed homo- and heterodimeric association among these three Arabidopsis DHNs, suggesting oligomerization to be a part of the LEA molecular mechanism. However, how client protein stabilization by LEA proteins occurs is still unclear. Therefore, we currently explore the interaction between LEA proteins and client enzymes through the analysis of sequences and structural characteristics that are responsible for enzyme stabilization. This study will provide an integrative view of LEA behavior during protein stabilization and contribute to the mechanistic understanding of cellular and organismic survival under extreme dehydration.

192. Autophagy is required for the hydrotropic response of *Arabidopsis thaliana*

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Hydrotropism is the tropism that involves the perception of water gradient and in consequence the root changes its growth direction to the water source. This process is considered an adaptive strategy to resist drought.

We reported that autophagy flux was required for root bending and that autophagosomes are accumulated in the bending zone. Remarkably, several *atg* mutants did not show hydrotropic curvature in low water potential gradient systems. These results suggest that autophagy is required for the hydrotropic response (Jimenez-Nopala, et al., 2018). Interestingly, Hyper, an H_2O_2 sensor showed that H_2O_2 preferentially accumulated in the root curvature at a similar rate as the autophagosomes did during hydrotropic response. Peroxidase and ROBH activity inhibition affected, negatively or positively root curvature. This data suggested H_2O_2 balance was required for root bending. Malondialdehyde, a metabolite used as an indicator of oxidative stress, accumulated at the same rate during the development of the curvature. We propose that the perception of water stress by *Arabidopsis* root induces H_2O_2 accumulation, which in turn promotes autophagy as a protective mechanism that allows the cell to degrade oxidized products during water stress.

Expression patterns of *AtATG8* gene family in response to a low water potential gradient showed that *AtATG8b* and *AtATG8i* are mainly expressed and that autophagy mutants in these genes did not show hydrotropic curvature compared with the wild type during water stress (Jiménez-Nopala et al., 2018). We will show differential accumulation of autophagosome during the hydrotropic response using a transgenic plants expressing *pATG8b:GFP:ATG8b* or *pATG8i:RFP:ATG8i* in the bending zone of the root. Results will be discussed related to non-redundant and specific expression of different ATG8 family members.

193. Functional characterization of the YTH domain protein ECT8 of *Arabidopsis thaliana.*

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Chemical modification of mRNAs has recently emerged as an additional and important layer in the control of gene expression. *N*^{6}-methyladenosine (m6A) is the most prevalent modified nucleotide in mRNA, with around 25% of mRNAs containing at least one m6A[1,2]. Similar to other eukaryotes, m6As in plant mRNAs are found within a sequence motif conserved across kingdoms and almost exclusively on exons, with a very strong enrichment in terminal exons and 3´untranslated regions[3-5]. m6A function is decoded by a group of proteins that share the YTH domain, which forms a hydrophobic pocket that directly accommodates the m6A residues. In *Arabidopsis thaliana*, YTH domain proteins belong to an expanded family of proteins called EVOLUTIONARILY CONSERVED C-TERMINAL REGION1-11 (ECT1-11). Recent evolutionary studies and publicly available microarray data analysis suggest that Arabidopsis ECT proteins are highly likely to be actual readers with redundant as well as specific functions. Here, we report the phenotypic characterization of an ECT8 (At1g79270) Arabidopsis mutant line, a member of the YTH-domain protein family whose gene expression is activated by leaf senescence and abiotic stress[6]. The T-DNA position in this mutant, named *ect8-1*, is in the 5′ UTR (untranslated region) of the ECT8 gene. Compared to wild type (Columbia ecotype), *A. thaliana* ECT8 mutants exhibit less sensitivity to abscisic acid (ABA) during seed germination and reduced plant growth on salt stress. The expression levels of ECT8 were determined in these experiments by quantitative real time PCR (qPCR). These data and other advances will be presented and thoroughly discussed.

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194. Characterization of plant innate immunity to *Botrytis cinerea* by chemical genomics

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Plants have developed several mechanisms to respond to the constant effect of biotic and abiotic stress. During the plant-pathogen interaction, reactive oxygen species (ROS) have been shown to play a versatile role, mediating stress responses, such as cell wall reinforcement and post-translational modification due to the oxidation of disulfide bonds in the cysteine residues. Because ROS impacts in multiple cellular processes, their study using traditional genetic screenings has not been fully successful. Here, we search for new molecular elements involved in ROS induction and regulation using chemical genetic screenings. The chemical genomics uses small molecules, which interfere with the functioning of proteins, thus allowing to avoid genetic redundancy and pleiotropic phenotypes. As a first approach, we develop a fast and robust bioassay to assess early ROS production using liquid cultures of *A. thaliana* seedlings placed in 96-well microtiter plates, which allow chemical intervention. Later, to identify bioactive molecules that modify ROS accumulation during the early immune response of *A. thaliana*, we carried out a chemical screening using 1600 molecules from LACTA library. To date, we have found 23 small molecules that impact in ROS accumulation in *A. thaliana*. This research work opens new scenarios on the mechanisms of ROS accumulation in plants.

195. Overexpression of a CpBI-1 type gene subjected to extreme temperatures in somatic embryos of *Carica papaya*

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Plant stress is estimated to reduce 70 per cent of global agricultural production. In order to solve this problem it is necessary to know the mechanisms of response of the plants to the abiotic stress. Stress is defined as the presence of a factor external to the plant caused by the changing environment, which exerts a negative influence on its optimal development. In plants a large number of genes are regulated in responses to abiotic stress. The BI-1 family genes play a very important role in the mechanism of tolerance to various types of abiotic stresses in plants, such as drought, salinity and low or high temperatures. BI-1 homologues have been identified in other plant species, except in *Carica papaya.* The objective of this work was to determine the effect of extreme temperatures on the overexpression of a CpBI-1 type of somatic embryos of Carica papaya. It was found that under the conditions studied it was possible to determine the application of temperature stress in the overexpression of the cpBI-1 gene of Carica papaya embryos, finding that the expression of this gene was inhibited by this abiotic stress.

196. Discerning the influence of biochemical and physical factors involved on oviposition site preference by *Anastrepha obliqua* through comparative metabolomic analysis in mango

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Anastrepha obliqua (Macquart), West Indian or mango fruit fly, is one of the most important mango pests in Mexico, Central and South America. It has been observed that female flies, before inserting eggs into the fruit, display several behaviors apparently evolved to evaluate fruit quality and select the exact oviposition site in the fruit. To address if biochemical and physical factors might be involved on oviposition site preference by *A. obliqua*, we performed an untargeted metabolomic analysis based in accurate mass spectrometry in three mango cultivars (Tommy Atkins, Manila and Criollo) to find distinguishing chemical markers that could be associated to these different behavioral observations. We found as chemical markers in Criollo, the most susceptible cultivar tested, mangiferin and quercetin, two phenolic compounds well-known by their beneficial properties. Our hypothesis is that the high susceptibility of Criollo to *Anastrepha* Fruit Flies attack is due the content of beneficial compounds that positively contribute to their development. Next we determined if light incidence and chemical factors influence the oviposition site preferences in a single Criollo mango. We found that *A. obliqua* females preferred to oviposit on the shaded areas and top level of mango fruit. Fruit flies oviposition preferences did not correlate to total levels of carbohydrates, lipid, protein and phenolic compounds and we found a highly variable pattern in specific phenolic compounds. A second untargeted metabolomics analysis was performed to find chemical factors involved in the oviposition preferences in Criollo mango and we found as biomarker in non-oviposited samples dihydrophaseic acid glucoside, a byproduct of abscisic acid (ABA) catabolism. ABA is one of the phytohormones involved in fruit ripening. Oviposition sites preferences are influenced by fruit chemicals as well as visual stimulus such as light incidence, which should also affect the chemical composition of mango.

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197. Initial characterization of knockout mutants of two *Physcomitrella* AP2-like genes

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Transcription factors (TFs) are master regulators which can control gene expression. In plants genome, approximately 7% of the coding regions correspond to TFs which include multiple members involved in early-responses to abiotic stress (Lindemose et al., 2013). The TFs classified into the APETALA-2 (AP2) family are involved in a variety of functions throughout the plant life cycle, for example: cell proliferation, vegetative and reproductive development, hormonal responses, and various types of biotic and abiotic stresses. Plant development and adaptation to different abiotic stresses involve the participation of the stress hormone Abscisic Acid (ABA). This phytohormone has been shown to cross-talk with sugars responses in vascular plants.
In nature, there are plants with the ability to tolerate different types of stress. Bryophytes represent the largest group of plants with desiccation tolerant species. *Physcomitrella patens* moss is considered a model stress tolerant bryophyte due to different properties that make it ideal for metabolic, phylogenetic and genomic studies.
Our group has focused efforts on the search for genes with homology to transcriptional factors AP2-like in *P. patens*. In this work, two sequences with homology to AP2 type genes were selected for functional characterization through knockout mutants. Here, we will present the initial characterization of knockout lines for both genes concerning their responses to osmotic, saline, ABA and glucose stress. Also, we tested the possible functional complementation of one of these genes in *Arabidopsis thaliana*.
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198. *In vivo* analysis of the interaction of LEA proteins of *Arabidopsis thaliana's* group 4

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LEAs (late embryogenic abundant) are intrinsically disordered proteins presented in the plant during water deficit stress. These proteins have been grouped into 7 groups, in the case of the model plant, *Arabidopsis thaliana,* the group 4 is small with 3 members (AtLEA4-1, AtLEA4-2, and AtLEA4-5). This group, in specially AtLEA4-2 and AtLEA4-5, can prevent enzyme denaturation under partial dehydration and freeze-thaw treatments. We have reported that the amino region of LEA4 proteins can fold in an α-helix structure in low water availability conditions and protects at the same level as the full protein. Interestingly, the carboxyl region of these proteins does not fold under these water conditions and is not able to protect. We observed through *in vitro* experiments that the protein AtLEA4-2 and AtLEA4-5 form oligomers, the same way the amino region forms oligomers too.

In this work, we hypothesized that complete protein and the amino region which have a predisposition to structure can interact *in vivo*. We made different constructions with the sequence of AtLEA4-2, AtLEA4-5, AtLEA4-5's amino region, and AtLEA 4-5's carboxyl region into two distinct destiny vectors specific to use bimolecular fluorescence complementation (BiFC). We transformed *Nicotiana tabaco* leaves and we observed that the full AtLEA4-5 is prone to oligomerize better than the amino and carboxyl region. This gives information about the structure-function relationship on group 4 of AtLEA.

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199. Single nucleotide polymorphism analysis of *Ditylenchus dipsaci* for identification of candidate effectors genes

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Parasitic plant nematodes are one of the main causes of important economic losses in agriculture worldwide. While research focuses on sedentary nematodes, root-knot and cyst ones, migratory nematodes such as *D. dipsaci* become a major risk given the peculiar characteristics, such as, diversity of pathogenic species, multiple host plants, and ease to adapt and survive extreme conditions. Knowledge about parasitism mechanisms, such as the production of effectors, is of great importance to generate strategies for the control and management of migratory nematodes. Single-nucleotide polymorphisms (SNPs) have become the focus of a large number of studies designed to identify critical differences in DNA sequence, which contribute to phenotypic variation for specific traits. Effector variation is crucial for the understanding of differencial virulence in plant-pathogen interactions. In this work, we present some results of the SNPs analysis in different strains of the *D. dipsaci*. Four different strains of *D. dipsaci*; Ags1, A5, Ags2 and B2 were analyzed. The strains were isolated from garlic bulbs (Ags1, A5, Ags2) and alfalfa (B2) and identified by PCR amplification of primers specific for *D. dipsaci*. DNA was sequenced through Illumina and Nanopore technologies and analyzed using bioinformatics tools. Sequences were aligned using a *D. dipsaci* draft genome sequence as a reference. SNPs filtering was performed using samtools mpileup and bcftools call. Several genes encoding putative effectors involved in parasitism have been identified. Our results contribute to the understanding of the mechanisms of action of the nematode in the infestation of agricultural products of economic importance for the region. This may aid the development of environmentally friendly solutions against this phytoparasitic nematode.

200. *IAA-LEUCINE-RESISTANT3* (*ILR3*) modulates root architecture reconfiguration of *Arabidopsis thaliana* in response to phosphate deprivation

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Phosphate (Pi) deficiency is a common agricultural problem in acid and alkaline soils. It has been correlated to iron toxicity, but little is known about the genetic components that modulate such crosstalk. Previous reports indicated alterations in iron uptake and homeostasis under low Pi growth conditions, which halt root elongation and promotes lateral root formation as an adaptive strategy. The bHLH leucine zipper transcription factor ILR3 regulates iron homeostasis; therefore, we evaluated its possible role in root organogenesis following Pi deficiency.
ILR3:uidA expression was inversely correlated with phosphate concentration in culture media. To get further insight into the possible causes that led to hypersensitive responses in *ilr3-1*, we evaluated auxin accumulation and transport by crossing *ilr3-1* with transgenic lines *DR5:GFP* and *PIN1:PIN1:GFP* and found an inhibited expression in both lines. This correlated with an increase in callose deposition in the primary root tip, which directly impacted meristematic activity. In addition, an alteration in the expression of the high affinity Pi transport *AtPT2:uidA* in the *ilr3-1* mutant background was observed under different combinations of iron and Pi deficiencies. Our results suggest the participation of *ILR3* in the modulation of the responses to phosphate deficiency, through the negative regulation of *AtPT2*, as well as a positive regulation in callose deposition, which modifies auxin levels at the primary root tip and impacts meristem maintenance.

Key words: *Arabidopsis thaliana, ILR3*, phosphate deficiency, iron deficiency

201. Evaluation of the response to phytoregulators of the *in vitro* culture of *Bacopa procumbens*.

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Bacopa procumbens is a repent Mexican plant commonly named as metatera, this plant produces phenolic and terpenic compounds that have cicatrization and antimicrobial effects, besides presenting the capacity to synthetizes noble metal nanoparticles. To achieve a continuous method for the obtention of these compounds we established the *in vitro* culture and evaluated its response to the cytokinis kinetin (KN) and 6-benzylaminopurine (BAP) in combination with the auxins indole-3-acetic acid (IAA), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) to obtain friable callus. The *in vitro* culture was obtained using nodal explants of greenhouse grown plants and the disinfecting protocol were established using a 15% solution of NaClO, being the best treatment for disinfecting the explants with a survival rate of 93.2%. Plantlets were grown using basal Murashige and Skoog (MS) medium, in the 1st week the explants developed roots and by the 4th week we obtained 11.96 ± 0.33 shoots per flask. Steams were taken in the 5th week to evaluate its response to the phytoregulators. Rhaman et. Al. in 2002 obtained friable callus in *B. monnieri* using NAA 1mg/L and KN 0.5 mg/L, instead we obtained long shoots with thick roots after 4 weeks. IAA in combination with BAP produced green callus in the 2nd week in all the concentrations proved, this callus become multishoot by the 3rd week being the longest shoots that obtained with IAA 2 mg/L and BAP 1 mg/L. In the achieve to obtain friable callus, we proved 2,4-D with BAP and these generated compact callus in all the concentrations proved, so we used NAA again but changing BAP instead of KN, and we obtained multishoot with NAA 1mg/L and BAP 0.5 mg/L and friable callus with NAA 3mg/L and BAP 1 mg/L. The multishoots obtained could be used for the propagation and conservation of the specie, and the callus are candidate to making cell suspensions and elicitation of the interest compounds for a continuous production.

202. Titanium dioxide nanoparticles increase the photosynthetic performance, but not the antioxidant system in *Solanum lycopersicum* L.

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Light response curve and antioxidant systems were evaluated by titanium dioxide nanoparticles (NPs TiO_2) applied to seeds of *S. lycopersicum*. Doses of 1000 and 2000 mg L^{-1} of NPs and 2000 mg L^{-1} of TiO_2 microparticles (μPs) and deionized water as control were used. All treatments of NPs TiO_2 were sonicated and stirred by 30 min each for adequate suspensions, immediately before seeds application on petri dish at 48 h for its imbibition. The seeds of each treatment were sown in individual pots with peat:perlite 3:1, irrigated by capillarity in a grown chamber at 25ºC, 55% RH, 200 μmol photons $m^{-2}s^{-1}$ source and UV-A light from led, and 16/8 h photoperiod. After 30 days of sowing, the light curves were measured by IRGA (LI-COR 6400 xt) and light compensation point (LCP) and light saturation point (LSP) were calculated. Leaves were harvested for biochemical analysis as photosynthetic pigments, lipid peroxidation (LP, through TBARS-method), total phenols (TP, by Folin-Ciocalteu-method) and antioxidant activity (AA, through DPPH-method). The main results showed a higher LCP and LSP by the effect of 2000 NPs and 2000 μPs TiO_2 respect to the control. In addition, the treatment of 1000 NPs reached a higher light curve over 1000 μmol photons $m^{-2}s^{-1}$ compared to the others treatments. In this context, Chl *a*+*b* and Chl *b* were higher at 1000 NPs compared to 2000 μPs, whereas, Chl *b* and carotenoids decreased at 2000 μPs. No differences were observed in Chl *a* and Chl *a*/*b*. A slight increase of antioxidant system seems to be observed at the highest dose, but without differences between the treatments. Finally, TiO_2 treatments, do not affect the antioxidant system, conversely, the photosynthetic performance measured through light response curve and Chl *a*+*b* and Chl *b* concentration was increased mainly in 1000 NPs on *S. lycopersicum*. **Acknowledgment:** VIPUCT N°201GI-CI-01 project and CONICYT-PCHA/Doctorado Nacional/2016-21160984

203. Effect of saline stress in *Bixa orellana* plants on the expression of the 9-cis-epoxycarotenoid dioxigenase (NCED) gene

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Bixa orellana (Achiote) is a perennial plant or small tree with a number of diploid chromosomes 2n = 14 of the Bixácea family which has six species, with a single Bixa genus. The most studied is *B. orellana* and its importance lies in the natural red pigment of its seeds consisting mainly of bixin and norbixin. It is used as an alternative among non-toxic natural dyes and in some products of the food, cosmetic, pharmaceutical and textile industry. Bixin is an apocarotenoid derived from carotenoids. Carotenoid biosynthesis begins with the condensation of two GGPP molecules in a reaction mediated by the enzyme phytoene synthase to form a 40 carbon molecule phytoen. Through desaturation and isomerization reactions, the phytoen molecule is transformed into lycopene, which is considered the main precursor of bixin biosynthesis. There are genes identified with the name of excision dioxigenase carotenoids (CCDs) whose function is to make cuts in the lycopene molecule and other compounds to generate new precursors that give rise to new metabolites in the plant. Studies carried out in plants grown under conditions of water stress have allowed the identification of gene expression as a response; specifically 9-cis-expoxy dioxygenases (NCED) reported in the transcriptome of *Bixa orellana* identified as a type of *CCDs* gene and the main precursor of abscisic acid (ABA) absent under normal culture. Likewise, the increase in ABA has been associated with the over-expression of the *NCED* gene. In plants, ABA is a metabolite synthesized mainly in the roots and they act in stomata to regulate the loss of water during perspiration in a water stress condition.
This work is focused on the study of the effect of saline stress by sodium chloride on the expression of the 9-cis-expoxy dioxygenase (NCED) gene because it is the main precursor of ABA. The study model was the adult achiote plants of three different variants; P12, N5 and N4 which were irrigated with different concentrations of NaCl. The results of this study in the three variants of *B. orellana*, show an increase in the expression of the *NCED* gene in the treatment with 100 mM NaCl compared to the control group. This result is consistent with plants other than *B. orellana* grown under the same water stress condition.

204. Comparative analysis between desiccation tolerant and sensitive *Selaginella* species

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Selaginella species as study models have relevance due to its phylogenetic position as one of the earliest divergent vascular clades and the large diversity of ecological niches that occupies. Some species are adapted to extremely arid conditions and have evolved vegetative desiccation tolerance (DT). To dissect the genetic components of DT, the present study has a comparative approach including tolerant and sensitive *Selaginella* species. The main objective is to identify molecular signatures at genome level and determination of regulatory networks associated to the acquisition of DT in *Selaginella*. For the establishment of accurate indicators to define critical stages during DT process we have implemented several methodologies to measure tissue damage and metabolism recovery. Some of the evaluated methods included electrolyte leakage and lipid peroxidation, RNA integrity, TTC test, maximum quantum efficiency (Fv/Fm) and photosynthetic activity measurements. Selected indicators allowed us to determine specific water contents when the tissue loses viability during dehydration, as well as the degree of recovery during the rehydration stage. Furthermore, the indicators represent a simple and robust way to compare the degree of tolerance using different drying rates or equilibrium points. The proposed methodology could be implemented to determine critical stages of DT in other plant models, even in the characterization of novel tolerant plant species.

205. Biochemical Characterization of a Mango Tau Class Glutathione S-Transferase implied on Response to Thermal Stress

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The Glutathione S-Transferases (GSTs) have the function of detoxification of hydrophobic compounds derived from cell membrane degradation as well as secondary metabolites generated by oxidative stress. Mango turns out to be an excellent study model because it is subjected to a hydrothermal treatment (TH), where the expression of a tau class GST (GSTU) was identified. However, the biochemical characteristics that allow mango GSTU (MiGSTU) to respond to thermal stress generated by TH are still unknown. The study hereby is aimed to perform an MiGSTU biochemical characterization to learn more about the role of this enzyme, involved in the response to thermal stress. An MiGSTU aminoacid sequence analysis was carried out, having a theoretical structural model obtained, identifying the possible interactions and structural characteristics that partly explain its implication in thermal stress process. The pure enzyme was obtained from its heterologous overexpression in *E. coli* BL21, by design of an overexpression construct encoding the enzyme, and purified by immobilized metal affinity chromatography (IMAC). Thermostability, optimal temperature and pH analysis were performed from the pure enzyme to know its biochemical characteristics. The protein overexpression was achieved in soluble form, using 0.4mM IPTG as an inducer of overexpression, in a 10 h induction time at 37 °C; the pure protein was obtained using a 0.5M imidazole concentration in IMAC. The thermostability tests, on the other hand, show a 45 °C melting temperature value (Tm), plus a higher specific activity at a 30 °C after incubation for 1h, as well as a 40 °C optimum temperature and an optimal alkaline pH of 8. Based on all of the above, it could be suggested that MiGSTU plays an important role in the response to thermal stress caused by TH. Keywords: Glutathione S-Transferase, thermal stress, thermostability, biochemical characterization.

206. Fibrillarin and RNA viruses: still a black box

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The interaction between RNA viruses and the nucleolus represents a whole field of study. In the nucleolus, multiple cellular processes takes place, such as gene silencing, cell cycle progression, senescence, ribosomal biogenesis and small nuclear RNAs, and many forms of stress responses. Nucleolar proteins, mainly B23, nucleolin and fibrillarin (FBL) are used by different types of viruses. FBL catalyzes the 2-O methylation using a ribonucleoprotein complex (RNP) and a C/D box snoRNA (SNORD) guide. In such way, FBL mediates (1) the methylation in rRNA, on ribosomal biogenesis; (2) the methylation in histone H2A, which augments chromatin accessibility and Pol I transcription; and recently (3) mRNA methylation, which regulates gene expression, however it has not been elucidated at what point of the mRNA life-cycle occurs. In addition, a novel ribonuclease activity was found in FBL 2 of *Arabidopsis thaliana* (AtFib2) in rRNA, suggesting an additional role on ribosomal biogenesis. Despite all these activities, the functionality of FBL with certain animal and plant viruses is limited to form a viral RNP complex and perhaps protecting the viral RNA from degradation. Interestingly, most viruses that interact with FBL have a (+) ssRNA genome type. It has been shown that RNA viruses are methylated to camouflage themselves as "self" in the cytoplasm, precisely where FBL is relocalized, and even SNORDs under stress. From this, we propose that FBL generates modifications on the viral RNA necessary to complete its infective cycle.

207. Expression of Enzymes of the Glutathione S-transferase Family in Response to Thermal Stress in Mango fruits

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Plants, being sessile organisms, have developed very efficient defense systems to cope with stress conditions, such as the glutathione S-transferase family (GSTs). As a consequence of oxidative stress due to high temperatures, compounds derived from cell membrane degradation are produced, which can also be mobilized in a non-catalytic manner by GSTs. An increase in the gene expression of GST family enzymes was observed in studies based on a transcriptome of mango fruits subjected to hydrothermal treatment (TH), suggesting their participation in response to heat stress. A total of 19 transcripts corresponding to GSTs of different classes (tau, phi, lamda, zeta, teta and DHAR, among others) were identified in this study. Moreover, their level of expression was determined compared to an untreated mango transcriptome. The tau and phi class genes expression, which are exclusive of plants, as well as the most abundant, was evaluated by means of qPCR, both of which were stimulated due to TH, presenting greater stimulation of the gene expression in the tau class, from 3 to 6 times more compared to samples without TH. These sequences presented characteristic domains for the binding of secondary metabolites and degradation products of cell membranes. A high conservation degree within each class was revealed by the corresponding amino acid sequences analysis, as compared with those reported for other organisms, mainly in the substrate binding (sites G and H) domains, which was also corroborated by means of a phylogenetic analysis. Thus, as indicated by all of the above, despite the redundancy of genes that code for these enzymes, some classes in particular perform specific functions to different stimuli in the plant.
Keywords: Glutathione S-transferases, thermal stress, gene expression, transcriptome.

208. Modulation of the peroxidase ZmPrx35 from insect-resistant maize endosperms (*Zea mays L.;* P84C3R) in response to mechanical and insect damage.

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Maize (*Zea mays L*.) is the largest staple crop produced worldwide. Mexico has been considered as the origin and diversification center of this cereal. Fifty-nine native varieties have been identified in this country. Most of them are still cultivated by smallholders with limited access to land and modern production resources. Postharvest primary insect pests, such as the maizeweevil (*Sitophilus zeamais)* and the large grain borer (*Prostephanus truncatus)* are responsible to up to 40% of global production losses, affecting mainly to smallholders from developing countries. Actual alternatives for pest management, such as the hermetic storage facilities and the application of chemical insecticides are often unaffordable to smallholders. In consequence, modern breeding programs have endeavored to develop insect-resistant varieties. However, the knowledge of mechanisms and bases of natural resistance is still limited. In recent years, phenolic acids and endosperm peroxidases have been identified as chemical bases of insect resistance in maize kernels. However, their role is still discussed. Studying the highly insect-resistant *Zea mays* variety P84c3 was found that more than 90% of the total POD activity was provided by a single enzyme, identified as B6T173_MAIZE (or ZmPrx35), a class III peroxidase. The role of this novel enzyme in the insect resistance remains unknown. The aim of this work was to identify the contribution of ZmPrx35 to the mechanisms and bases of insect resistance displayed by kernels from some maize varieties and landraces. Interactions between insect-resistant endosperms (P84C3R) and the storage pests *S. zeamais* and *P. truncatus* demonstrated that ZmPrx35 activity and expression is modulated by mechanical andinsect damage, suggesting a contribution of this enzyme in the mechanism of antixenosis (repellence) for *S. zeamais*. In the case of *P. truncatus*, this pest inhibited kernel POD activity, suggesting pest adaptation to POD/phenolic defence mechanism. Our findings will contribute to the screening for insect resistant maize varieties and will support marker-assisted breeding and the identification of natural insect repellents.

209. *lincDUF* possible regulatory role over its *loci* in water deficit conditions

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Abiotic stress in plants produces a general physiological response, thus, they have evolved protective mechanisms to ensure their survival when are threatened by adverse environmental conditions. Hence, it is essential the study of the molecular basis underlying plant response to stress. Since, long non-coding RNAs (lncRNAs) participate as regulatory molecules, and involved in gene regulation in *Arabidopsis thaliana*. We aim to study the regulatory role of intergenic lncRNA's (*lincDUF)* over its *loci* in *A. thaliana* genome. For the moment, we are generating different tool to analyze the *lincDUF* effect over its *loci* as overexpression lines and mutant lines for further analysis. Since bioinformatic analyzes show that *lincDUF* and its neighbor genes are expressed under stress conditions such as salinity, drought and exogenous abscisic acid treatments. We are speculating that *lincDUF* maybe, having regulatory function on its surrounding genes under adverse conditions.

210. Contribution of maize germ in resistance to *S. zeamais* and *P. truncatus*.

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Maize (*Zea Mays*) is the most important cereal in terms of production with nearly 1,045 million tons produced worldwide. It's mainly cultivated by smallholder farmers due to its adaptability, high yields and valuable by-products. Postharvest losses caused by insect pests represent 12-36% worldwide. In Mexico, the main postharvest insect pests that affect maize are the maize weevil (*Sitophilus zeamais*), the large grain borer (*Prostephanus truncatus*) and the angoumois grain moth (*Sitotroga cerealella*). In vulnerable zones, losses caused by *S. zeamais* and *P. truncatus* can be as high of 80%. Natural resistance has been explored as a sustainable pest management alternative, and is mainly influenced by anatomical, biochemical and genetic factors that act through a mechanism of antibiosis or antixenosis.

Factors attributed with resistance of maize kernel against insect pests had been found in tissues such as pericarp, aleurone layer and endosperm; however, one structure that hasn't been studied in this area is germ. Thus, aim of this study is to understand the contribution of the maize germ to the resistance against *S. zeamais* and *P. truncatus*. This contribution was assessed by performing 60-days bioassays, testing both pests, 14 genotypes and different combinations of kernel structures: the whole kernel, the kernel without pericarp, the endosperm, and the germ.

Our findings provide strong evidence of an important contribution of the maize germ in the resistance against *S. zeamais* and *P. truncatus* by an antibiosis mechanism. Although oviposition was observed after 60 days, there was no evidence of adult or larvae emergence of both pests, suggesting a larvicidal or detrimental effect for the egg-plugs.

211. Aluminum induces cross-tolerance in suspension cells of *Capsicum chinense* to *Pythium ultimum*

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In Mexico, the genus *Capsicum* constitutes an important crop; however, its production is threatened by different pathogenic organisms such as *Pythium ultimum,* a necrotrophic oomycete responsible for a variety of diseases on a broad range of crops. Recent evidence suggests that plant exposure to one abiotic stress leads to acquired resistance to another stress, generating cross-tolerance. Aluminum can be toxic for some plants and pathogenic microorganisms; thus, we analyze the effect of aluminum on the resistance of a cell suspension culture of *C. chinense*, to *P. ultimum*. To address this question, the cell suspensions culture was treated with different concentrations of AlCl3 at pH 4.3 and then challenged them with an oospores suspension of *P. ultimum*. Two parameters were evaluated: cell integrity and aluminum location using mapping techniques (confocal and electronic microscopy-EDS), as well as, the expression levels of PR defense gene. It was evident the accumulation of aluminum in the cell nucleus, the tolerance at the infection and the overexpression of defense genes with pretreatment with 500 µM AlCl3 suggesting tolerance to infection acquired by multiple stress. This study allows to elucidate aspects of cross-tolerance and provides data for future genetic improvement strategies.

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212. SnRK1 phosphorylates PHR1, a key factor in phosphate starvation responses

Trejo-Fregoso, J., Salazar-Sosa, J., Guerrero, F., Coello, P.

Phosphorus (P) is one of the most important nutrients present in soils, but the assimilated form, inorganic phosphate (Pi), usually is in low quantities in the rhizosphere. To cope with these conditions, plants have developed several phosphate starvation responses (PSR) in order to optimize and increase Pi acquisition. The low phosphate conditions alter ATP synthesis and energetic homeostasis. Plant responses to energy stress are mediated by SnRK1, which is a protein kinase related to the yeast SNF1 and mammalian AMPK. Previous work in our lab showed differential regulation of SnRK1 catalytic subunits in plants growing in low Pi conditions and direct participation of these kinases in the regulation of gene expression and carbon metabolism. In this work, we identified potential targets of the SnRK1 involved in PSR, using synthetic peptides. Between these targets of SnRK1, we highlight PHR1, which is a transcription factor that regulates the expression of genes in response to Pi starvation. Protein-protein interaction assays revealed that the catalytic subunits of SnRK1 and PHR1 interact *in-vitro* and *in-vivo* and phosphorylation assays revealed that Ser11 is the primary phosphorylated residue in PHR1. Here we discuss the potential effects of this phosphorylation on PHR1 activity. Funding: PAPIIT IN227019

213. Tisular and intracellular localization of proteins induced by water deficit in *Arabidopsis thaliana*: group 4 LEA Proteins

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Late Embryogenesis Abundant (LEA) proteins accumulate during the last stages of seed development and in vegetative tissues in plants under water deficit. Previous work uncovered the relevance of group 4 LEA proteins in plant tolerance to water deficit. In *Arabidopsis thaliana* this group is formed by three members (*AtLEA4-1, AtLEA4-2 y AtLEA4-5*) and it was shown that mutant plants deficient in any of these members are more susceptible to water deficit; also, it has been demonstrated by *in vitro* assays that AtLEA4-2 y AtLEA4-5 proteins can protect the activity of reporter enzymes when they were subjected to partial dehydration or freezing/defrosting cycles. These data indicate that the proteins of this family could be exerting a protective function in the plant. Although it is known that there is a positive correlation of the accumulation of these proteins and the presence of water deficit, little is known about their function and localization in plant. In order to carry out a comparative analysis, it is proposed to address in this project the localization of the three members of this group in the plant, since it is relevant to know if its function is limited to certain tissues and cellular compartments during the water deficit response, if its localization changes according to the stage of development, and if the three proteins have a similar localization for each member. By using translational fusions of these proteins to GFP, so far we know that AtLEA4-5 protein is localized in primary and lateral roots, more abundantly in vascular tissues of seedlings subjected to water deficit; whereas in the aerial part is detected in leaf primordia. During embryo maturation it is localized in all the embryo, and during germination predominantly in radicle. The information obtained from this analysis could also indicate us in which biological processes in the plant are involved this proteins, as well as contribute to answering another questions, such as the fact that the phenotypes observed in the mutant plants in these members are due to their differential localization, although it is proposed that they could exert similar functions in the plant.
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214. Phenotypic and molecular response to biotic stress by geminiviruses in chili plants treated with PGPR

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Application of PGPR´s, isolated from the rhizosphere of guava orchards, in young plants of *Capsicum annuum* L. were performed, to evaluate role of these bacteria as elicitor in induced systemic resistance (ISR) leading to tolerance and/or resistance to stress biotic caused by mixed inoculation of geminivirus in this system. Two strains of Bacillus were evaluated and were applied both foliar and directly to the substrate, and subsequently inoculated by bioinjection with a mixture of geminiviruses. The viral mobility of mixed inoculations of geminiviruses in plants induced with PGPR´s was evaluated as well as the molecular response to biotic stress through the RTPCR analysis of genes related to oxidative stress and biotic stress: CAT, PR1, npr1, Mn-SOD, PAL and B-Act. Finally, our result shows that of the treatments applied, the concentration PGPR´s of 0.6 of OD show a higher expression of genes related to tolerance to biotic stress. With the analysis of the results it could be inferred that thePGPR is useful as a probable inducer of the *Capsicum annuum* plant's defense system.

215. Eukaryotic initiation factors 4E and the plant response to freezing stress

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Plants have evolved to develop mechanisms that allow them to contend with adverse environmental conditions; among these mechanisms, the regulation of gene expression plays a fundamental role. We aim to elucidate the role of translation initiation factors 4E (eIF4E and eIF(iso)4E) in the plant response to freezing stress and acclimation. Previously, our working group observed that the overexpression of these factors in *Arabidopsis thaliana* induces an increased resistance to freezing temperatures compared with WT and eIF(iso)4E mutant plants, this response seems to be influenced by the specialized translation of transcripts derived from genes involved in cryoprotection. However, the absence of eIF4E had not been explored. The objective of this work is to evaluate eIF4E mutants (SALK 145583 and CRISPR-Cas9 [currently in development by our laboratory]) in order to elucidate the differential role of eIF4E proteins in freezing stress response and corroborate our previous observations. This work was supported by CONACyT 238439 and PAPIIT IN214118.

216. Post-translational regulation of the zinc-finger transcription factor STOP1 underlies root adaptation to acidic soil conditions

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Acidic soil conditions constrain plant growth and development in both natural and agricultural ecosystems because of the increased levels of toxic cations, like Al3+, H+ and Mn2+, together with low Pi availability. The zinc-finger transcription factor SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) is known to underlie root adaptation to low pH, Al3+ toxicity and low Pi availability by activating the expression of multiple genes, including genes involved in organic acid exudation, the regulation of pH homeostasis, Al3+ detoxification and the modification of root architecture. However, the mechanism, or mechanisms, that trigger the pleiotropic response of this transcription factor to the common stresses present in acidic soil remains unknown. By using a coupled analysis of two independent translational fusions of STOP1 to mCherry and β-glucuronidase, we show that the level of STOP1 is regulated by a post-translational mechanism. The level of STOP1 increases, without a significant change in its gene expression level, in the roots of seedlings exposed to acidic conditions (pH 4.2), presence of toxic levels of Al3+ and low Pi availability. Our results indicate that protein-protein interactions between STOP1 and an unknown partner underlie root adaptation to acidic conditions in the medium. Here we explain the tandem affinity purification strategy coupled to mass spectrometry analysis that we are currently undertaking to characterize the landscape of STOP1 protein-protein interactions in order to provide insights into the molecular mechanisms underlying root adaptation to acidic soil conditions.

217. eIF4E family-mediated responses to abiotic stress in *Arabidopsis thaliana*

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Extreme temperatures cause damage to plants at different biological levels, but there are several molecular mechanisms allowing them to contend with these injuries. One important strategy involves expression regulation of genes associated with acclimation processes. In our laboratory we have explored the role of *Arabidopsis thaliana* eIF4E and eIFiso4E translation initiation factors in response to freezing temperatures finding that overexpression or lack of these proteins modifies stress tolerance when compared to wild type plants. For another eIF4E family member, nCBP, it was observed at subcellular level differential localization in cytoplasmic granules in response to several types of abiotic stress. In order to determine the molecular function of *Arabidopsis* eIF4E proteins, the aim of this work was to evaluate whether each mutant line shows distinct responses to low and high temperatures. Because members of the eIF4E family are recognized as crucial for viral infection by the Potyvirus family, *Arabidopsis thaliana* mutant lines carrying a synthetic modified eIF4E allele were generated to prevent recognition by virus. This mutant was included in the abiotic stress analyses to elucidate whether the design of eIF4E synthetic alleles resistant to viral infection might result in impairment of plant acclimation to extreme temperatures. This work was supported by CONACyT 238439, PAPIIT IN214118 and Facultad de Química PAIP 5000-9118.

218. Changes in root system architecture evoked by lead are regulated by phosphate in a *LPR1;2* dependent way

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In order to explore the soil, plants modify their root system post-embryonic development program (PEDP). In soil, in addition to water and nutrients, there are toxic elements such as heavy metals (HMs), among them lead (Pb). We have seen that when *Arabidopsis* grows in Pb stressor media MS 0.1 X (800µM Pb, 500 Pi µM) the lateral root density (LRD) increase and primary root length (PRL) decrease. Similar phenotype has been reported in phosphate (Pi) starvation (1 µM). We analize both Pb stress and phosphate starvation and we found that the primary root meristem is exausted, the QC cells identity and mitotic activitie are lost and cytokinin and auxin concentration reduce drasticaly in both treatments and also we found similar *AtPT1::GUS* and *AtPT2::GUS* expresion and organic acid exudation. *In silico* analysis show that Pb and Pi form an insoluble compound suggesting that effect of Pb on root phenotype could be related with Pi starvation. In order to determine if some of genetic pathway that regulate Pi starvation response are comparted by Pb stresss and Pi stavation responce, we grown *Arabidopsis* mutants insensitive to Pi starvation (*lpi3*, *lpr1;2* and *stop1*) on a Pb suplemented media. We found that *stop1* mutant show decrease in PRL and increse in LRD while *lpr1;2* and *lpi3* show resistance to Pb effects. Suggesting that the phenotype of evoked by Pb is dependent to *LPR1;2* showing a cross-talk between Pb-Pi responses. In order to avoid Pi starvation stress due to Pb-Pi coprecipitation, Arabidopsis seedlings were grown on 800µM Pb, aded with excess of Pi (1.0, 1.5, 2.0 and 10 mM). Pi excess (10 mM) rescue partially (60%) PRL and QC identity and cellular division index are recovered probably by an increase of auxins and cytokinin concentration in root meristem. Our result sugest that the Pb stress could be modulate by Pi in a *LPR1;2* dependent way.

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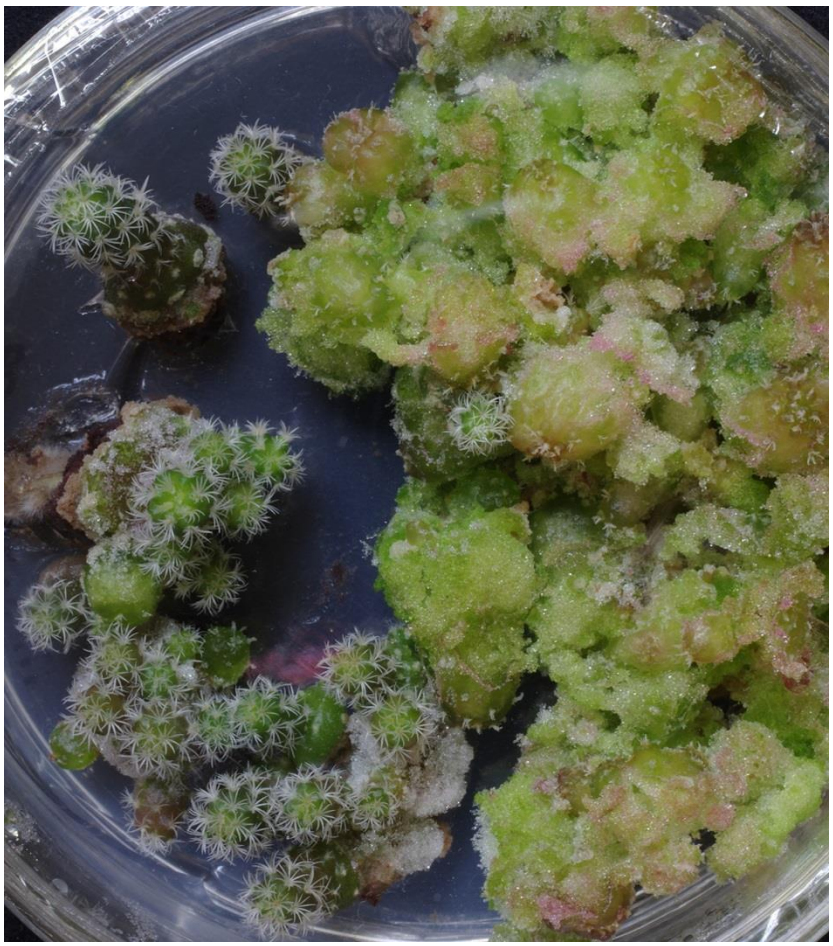

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Cover image: Abaxial leaf surface of *Strelitzia reginae.* Page 1: The opening. Pages 6 and 25: Inflorescence and flowers of *Agave victoriae-reginae.* Page 63: A tumorous formation on stems of *Neobuxbamia tetetzo* in Tehuacán-Cuicatlán Biological Reserve. Page 284: Plant regeneration in a tissue culture of a Cactaceae (reproduced with permission of Felipe Hernández Bermúdez and Svetlana Shishkova). This page: *Echinocactus platyacanthus*